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Plasticity of Human Spinal Locomotor Circuitry

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Summary

Neuronal circuits within the spinal cord can produce rhythmic locomotor movements. Fed by appropriate afferent input, these networks are capable of producing well-modulated rhythmic locomotor electromyographic (EMG) activity even after a complete spinal cord injury (SCI) during assisted locomotion within a driven gait orthosis. In chronic (> one year after SCI) complete SCI subjects an exhaustion, i.e., a drop of leg muscle EMG amplitude occurs over time during assisted locomotion. This exhaustion phenomenon was attributed to a degradation of spinal interneuronal function. The preservation of spinal interneuronal function is, however, a prerequisite for the success of any future regeneration-inducing therapies. Therefore, this thesis investigated the changes in the function of spinal neuronal circuits over the course after an SCI in humans with the goal to develop countermeasures. The behaviour of the spinal neuronal circuits was studied by analysing leg muscle EMG underlying locomotion and spinal reflex (SR) function after an SCI. In healthy subjects the SR evoked by non-noxious tibial nerve stimulation consists of an early (latency 60 - 120 ms) SR component in the ipsilateral tibalis anterior muscle.

Changes in SR in complete SCI subjects and the relationship between SR and locomotor activity were analysed by the first study: At about one year after an SCI, i.e., around the time when the EMG exhaustion phenomenon emerges, the amplitude of the early component decreases and a second, late SR component (latency 120 - 450 ms) appears. In the chronic stage of an SCI, only a late SR component dominates, combined with full expression of the EMG exhaustion phenomenon. A temporal relationship exists between the shift from dominant early SR to dominant late SR component and the degree of exhaustion of locomotor activity. Therefore, it was assumed that common neuronal mechanisms underlie the changes in SR behaviour and locomotor activity after an SCI.

Arguments for common neuronal pathways between SR and spinal locomotor circuitries were provided by clarifying whether SR function can be used as a neurophysiological marker for the functional state of spinal locomotor circuitry. The second study investigated the relationship between walking capacity and SR behaviour after SCI. The main observations were: The better the walking capacity of SCI subjects is, the more dominant is the early SR component reflecting a functional state of spinal locomotor circuitry. In addition, the explotion of the plasticity of these neuronal networks by a

locomotor training became demonstrated: An intense locomotor training over one month lead to a gain in walking capacity associated by a strengthening of the early SR component in severely affected, but incomplete SCI subjects. In contrast, chronic motor complete SCI subjects cannot improve their walking capacity by such a locomotor training and the late SR component stays dominant.

In a further step the function of the early and the late SR components was explored. The third study analysed the role of the two different SR components during assisted locomotion in complete SCI subjects. The interaction of the two SR components with the locomotor EMG pattern induced by Lokomat stepping was analysed. The main observations were: The early and late SR components have a differential effect on the locomotor EMG pattern, i.e., a 'normal' compensatory reaction by the early and a long-lasting disruption of the pattern by the late SR component. Therefore, the early SR component was assumed to reflect a largely "preserved" function of spinal locomotor circuitries (see also studies 1 and 2). In contrast, the dominance of a late SR component was assumed to reflect a dysfunction of spinal neuronal circuits below the level of lesion deprived of supraspinal input.

Since spinal locomotor circuitries are influenced by supraspinal drive, not only SCI subjects but also hemiparetic stroke survivors experience impaired locomotion. Thus, the fourth study investigated to what extent a neuronal dysfunction, corresponding to that described in SCI subjects develops in severely affected stroke subjects: Similarly to SCI, a shift from an early to a dominant late SR component develops in the affected leg of severely disabled stroke survivors. This shift is, similar as in SCI subjects, related to the walking capacity. However, in contrast to the condition after SCI, this shift is not associated with an EMG exhaustion phenomenon during assisted locomotion in stroke subjects. Hence, lesion-dependent effects on spinal neuronal dysfunction after the loss of supraspinal drive can be assumed.

In conclusion it has been shown that a severe SCI leads to a dysfunction of spinal neuronal circuits underlying locomotor activity and the associated SR behaviour. The SR can be used as a marker for locomotor dysfunction and the state of spinal neuronal circuits underlying locomotion. In incomplete SCI subjects first approaches have been proposed to overcome a spinal neuronal dysfunction by a functional training. An intensive locomotor training can positively influence both the locomotor function and the SR behaviour.

Zusammenfassung

Neuronale Schaltkreise im Rückenmark können rhythmische Lokomotionsmuster produzieren. Bei adäquaten afferenten Signalen von den Gliedmassen während assistierter Lokomotion sind diese Schaltkreise auch nach einer kompletten Querschnittlähmung fähig, modulierte rhythmische elektromyographische (EMG) Aktivität zu produzieren. Bei Patienten mit einer chronischen kompletten Querschnittlähmung erfolgt jedoch eine Abnahme dieser EMG Aktivität in der Beinmuskulatur während assistierter Lokomotion. Dieses Ermüdungs-Phänomen wurde auf eine gestörte Funktion spinaler Interneuronen-Schaltkreise zurückgeführt. Für zukünftige Regenerationsansätze ist es jedoch notwendig, dass diese spinalen Interneuronen-Schaltkreise funktionsfähig bleiben. In dieser Dissertation wurden die Veränderungen in spinalen neuronalen Schaltkreisen im Verlauf einer Querschnittlähmung im Menschen untersucht, mit dem Ziel, Gegenmassnahmen zu entwickeln. Einsicht in das Verhalten dieser spinalen neuronalen Schaltkreise bieten zum einen Analysen der Beinmuskelaktivität bei der Lokomotion und zum anderen Messungen von spinalen Reflexen. Bei gesunden Probanden tritt der spinale Reflex, ausgelöst durch nicht schmerzhafte elektrische Stimulation am Nervus tibialis, als eine frühe Komponente (Latenz 60 - 120 ms) im Musculus tibialis anterior des stimulierten Beines auf.

In der ersten Studie wurden Lokomotionsaktivität und spinale Reflexe bei Patienten mit kompletter Querschnittlähmung untersucht. Ungefähr ein Jahr nach einer Querschnittlähmung, zum Zeitpunkt wenn das EMG Ermüdungs-Phänomen beginnt, nimmt die Amplitude der frühen Reflexkomponente ab und eine zweite späte Reflexkomponente (Latenz 120 – 450 ms) tritt auf. Im chronischen Stadium einer Querschnittlähmung dominiert die späte Reflexkomponente, kombiniert mit der vollen Ausprägung der EMG Ermüdung während assistierter Lokomotion. Es besteht ein zeitlicher Zusammenhang zwischen dem Wechsel von dominanter früher zu dominanter später Reflexkomponente und dem Ausprägungsgrad der Erschöpfung der Lokomotionsaktivität. Gemeinsame neuronale Mechanismen scheinen diesen Veränderungen in spinalen Reflexen und Lokomotionsaktivität nach einer Querschnittlähmung zu Grunde zu liegen.

Die zweite Studie untersuchte den Zusammenhang zwischen der Gehfähigkeit und dem Verhalten spinaler Reflexe. Je besser die Gehfähigkeit der Patienten mit Querschnittlähmung ist, desto dominanter ist die frühe Reflexkomponente, d.h. diese spiegelt den funktionellen Status spinaler Lokomotionszentren wider. Bei Patienten mit

stark ausgeprägter inkompletter Querschnittlähmung führt ein intensives Lokomotionstraining über einen Monat zu einer besseren Gehfähigkeit und zu einer verstärkten frühen Reflexkomponente. Im Gegensatz dazu kann bei chronischen Patienten mit kompletter Querschnittlähmung die Gehfähigkeit durch ein Lokomotionstraining nicht verbessert werden und die späte Reflexkomponente bleibt dominant.

Die dritte Studie untersuchte die Funktion der frühen und späten Reflexkomponente in Bezug auf die Lokomotionsaktivität bei Patienten mit kompletter Querschnittlähmung. Es wurde die Wechselbeziehung zwischen den unterschiedlichen Reflexkomponenten und der Lokomotionsaktivität während assistierter Lokomotion im Lokomat analysiert. Die frühe Reflexkomponente hat eine ‚normale' kompensatorische Reaktion und die späte Reflexkomponente eine langanhaltende Störung der Lokomotionsaktivität zur Folge. Daher wurde der frühen Reflexkomponente eine aufrecht erhaltende Funktion spinaler Lokomotionszentren zugesprochen. Im Gegensatz dazu wurde angenommen, dass die späte Reflexkomponente einen antagonistischen Effekt auf spinale Lokomotionszentren hat.

Spinale Lokomotionszentren stehen unter supraspinaler Kontrolle, daher ist die Lokomotion nicht nur nach einer Querschnittlähmung, sondern auch nach einem Schlaganfall beeinträchtigt. In der vierten Stude wurde untersucht, in welchem Ausmass sich eine spinale neuronale Dysfunktion nach schwerem Schlaganfall entwickelt. Ähnlich wie nach einer Querschnittlähmung entwickelt sich im betroffenen Bein von Patienten mit schwerem Schlaganfall eine Verschiebung von einer dominanten frühen zu einer dominanten späten Reflexkomponente. Diese Veränderung der Reflexkomponenten ist, wie bei Patienten mit Querschnittlähmung, mit der Schwere der Gangstörung verbunden, jedoch nicht mit einem EMG Ermüdungs-Phänomen während assistierter Lokomotion. Diese Tatsache weist auf einen läsionsabhängigen Effekt von gestörtem supraspinalem Einfluss auf die Funktionsveränderungen spinaler neuronaler Schaltkreise hin.

Zusammenfassend wurde mit dieser Dissertation gezeigt, dass sich nach einer schweren Querschnittlähmung eine Dysfunktion spinaler neuronaler Schaltkreise, welche der Lokomotionsaktivität und assoziierten Reflexen zu Grunde liegen, entwickelt. Spinale Reflexe können als Marker für Gangstörungen und den funktionellen Status spinaler Lokomotionszentren genutzt werden. Bei Patienten mit inkompletter Querschnittlähmung konnten erste Ansätze zur Bewältigung einer spinalen neuronalen Dysfunktion vorgeschlagen werden. Ein intensives Lokomotionstraining kann sowohl die Gehfähigkeit, als auch das Verhalten spinaler Reflexe positiv beeinflussen.

1 General Introduction

1.1 Spinal cord injury

The spinal cord is the part of the central nervous system executing most autonomically performed movements such as locomotion, receiving sensory information from all over the body, interacting with the environment and controlling autonomic functions. Damage of the spinal cord, for example, a spinal cord injury (SCI) leads to a loss or impairment of sensori-motor function, chronic pain syndrome, bladder, bowel and sexual dysfunction. The incidence of SCI is, compared to stroke, rather small (Feigin et al., 2003, Wyndaele and Wyndaele, 2006) and varies between 10.4 and 83 per million inhabitants per year world-wide (Wyndaele and Wyndaele, 2006). The mean age of subjects experiencing an SCI is 33 years and men are more affected than women (3.8 to 1) (Wyndaele and Wyndaele, 2006). Not only personal consequences but also economical costs of spinal cord damage are high.

For most of the SCI subjects, bladder and bowel control, and for tetraplegic subjects upper extremity functions are the main focus of concern and interest to become restored (Anderson, 2004). Nevertheless, independent from time after lesion, age at time of injury and severity of the SCI, restoration of motor function including walking ability is also of high priority for SCI subjects (Ditunno et al., 2008).

1.1.1 Classification of spinal cord injury

An SCI is generally classified by the completeness and the neurological level of lesion. The American Spinal Injury Association (Marino et al., 2003) established the "International Standards for Neurological and Functional Classification of Spinal Cord Injury" for a standardised assessment of spinal cord function. Sensory function is tested as light touch and pinprick sensation at defined dermatomal key points on both body sides. The sensitivity is scored as 0 (absent), 1 (impaired) or 2 (normal). Motor function of different segments is tested in 10 key muscles and graded from 0 - 5 (from total paralysis to active movement against full resistance). The most caudal segment of the spinal cord with normal motor and sensory function is then defined as the neurological level of lesion. The completeness of injury is graded from A to E, see Tab. 1.1.

Classification	Functional impairment
A = Complete	No sensory or motor function is preserved in the sacral segments S4-S5.
B = Incomplete	Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5.
C = Incomplete	Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3 (Grades 0-2).
D = Incomplete	Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade greater than or equal to 3.
E = Normal	Sensory and motor function is normal.

Tab. 1.1: Classification of spinal cord injury according to the American Spinal Injury Association (ASIA) Impairment Scale (AIS) (Marino et al., 2003).

1.1.2 Assessment of locomotor function after spinal cord injury

Locomotor neuronal function after SCI can be quantified by electromyographic (EMG) recordings during prolonged assisted or free walking conditions. The ASIA motor score assesses muscle strength which has been shown to correlate with walking speed (Kim et al., 2004). Other measures for locomotor function are the 10 meters walking test (10MWT) (van Hedel et al., 2008), the Walking Index for Spinal Cord Injury (WISCI II) (Ditunno et al., 2000) and the Spinal Cord Independence Measure (SCIM, mobility part) (Catz et al., 2001). In addition, there are several well established neurophysiological measurements to assess the prediction of locomotor motor outcome after an SCI, such as somato-sensory evoked potentials (SSEPs) to assess afferent impulse conductivity of spinal tracts, as well as motor evoked potentials (MEPs) to assess corticospinal tract function (Zorner et al., 2010).

1.2 Spinal locomotor circuitries

Neural circuits in the spinal cord, called central pattern generators (CPGs), can produce locomotor movements (Grillner et al., 1981, Kiehn, 2006). The term CPG reflects rhythmic activity generated in completely spinalised and paralysed animals. Under this condition the input from the periphery and from descending pathways from the brainstem

and the telencephalon is removed. Therefore, the rhythmic activity induced, e.g., by brainstem stimulation, can only be of spinal origin and is termed “fictive locomotion”. However, in real life conditions locomotion includes starting, turning, accelerating, decelerating, and stopping. Hence, the spinal locomotor circuitries interact continuously with various descending supraspinal pathways and peripheral feedback information (Rossignol et al., 2006). For example, the reticular formation in the brainstem plays a crucial role in initiation of locomotion, while the motor cortex controls various adaptive functions associated with locomotion, such as gait modification over an obstacle (Armstrong, 1986, Drew et al., 2004).

1.2.1 Physiological basis - animal model

So far, the neuronal organisation of spinal locomotor circuitries has been characterised in lower vertebrates, i.e., the lamprey and the tadpole. The core of this network consists of excitatory glutamatergic and inhibitory glycinergic interneurons. The glutamatergic interneurons project ipsilaterally and provide the excitatory drive necessary to produce sustained rhythmic locomotor activity, while the glycinergic interneurons project to the contralateral side and mediate the reciprocal inhibition responsible for the alternating activity between the two sides of the spinal cord (Buchanan, 2001, Grillner, 2003, Roberts et al., 1998, Sillar et al., 1998).

In mammals, including rats, mice and cats, the locomotor pattern with flexion and extension coordination at different joints of one leg and the interlimb coordination requires a more complex organisation of spinal locomotor circuitries. In rodents both descending glutamatergic reticulospinal projections and descending serotonergic projections located in the hindbrain are responsible for the activation of the spinal locomotor circuitries and produce basic aspects of locomotion: (i) rhythm-generation, (ii) left-right coordination, and (iii) flexor-extensor alternation (Kiehn, 2006). The following parts will outline the lumbar spinal circuits responsible for these basic aspects of hindlimb movements in rodents.

Network pharmacology and lesion studies have revealed that the rhythm-generating neurons are glutamatergic excitatory interneurons with ipsilateral projections to motoneurons, i.e., EphA4 (EphrinA4) positive neurons (Butt et al., 2005) and the Hb9 (homeobox 9) positive neurons (Hinckley et al., 2005).

The spinal neurons that are directly involved in the left-right coordination during locomotion are commissural interneurons which project inter- and intrasegmentaly

(Stokke et al., 2002). The intersegmental pathway includes rostrally located descending commissural interneurons and link flexor and extensor motoneurons diagonally across the spinal cord into synergies (Kiehn et al., 2010). On the other side the intrasegmental pathways reflect a dual inhibitory system (Quinlan and Kiehn, 2007). This dual inhibitory system acts directly via inhibitory glycinergic/GABAergic commissural interneurons on contralateral motoneurons, or by excitatory glutamatergic commissural interneurons providing indirect inhibiton of contralateral motoneurons via inhibitory Renshaw cells. During synchronous activity of left and right site a single excitatory glutamatergic system might activate motoneurons (Quinlan and Kiehn, 2007).

Locomotion in rodents requires an alternating activity of flexor and extensor muscles within a limb. Since flexor and extensor muscles work in synchrony when all inhibitory transmission is blocked during locomotion (Nishimaru and Kakizaki, 2009), it is assumed that inhibitory networks allow a rhythmic activation of flexor-extensor motoneurons. These inhibitory networks are not well defined yet, but are assumed to project ipsilaterally because even in the hemicord flexor-extensor alternation persists (Kiehn, 2006).

1.2.2 Human spinal locomotor circuitry

In contrast to animal models, the most convincing evidence for a CPG, i.e., “fictive locomotion”, has no direct equivalent in humans. From an evolutionary point of view, however, locomotor generating networks have to be conserved and integrated in bipedal gait and greater corticalisation. There is evidence for the existence of human spinal locomotor circuitries capable of generating the basic locomotor pattern. This evidence rises mainly from observations in complete SCI subjects (Dietz et al., 1995, Duysens and Van de Crommert, 1998). Early descriptions of involuntary step-like movements in complete paraplegic subjects date back to the work of Lhermite (1919). Later on, alternating myoclonic activity was observed in complete paraplegic subjects after hip extension (Bussel et al., 1988, Calancie, 2006). This rhythmic activity could be stopped, initiated and modulated by peripheral stimulation of flexor reflex afferents (Bussel et al., 1989). Further, epidural electrical stimulation of the spinal cord was able to induce rhythmic activation of leg flexor and extensors in complete SCI subjects (Dimitrijevic et al., 1998, Minassian et al., 2004, Rosenfeld, 1995). Additional indirect evidence for spinal locomotor circuitries were suggested by studies inducing well-modulated locomotor EMG pattern in motor complete SCI subjects during stepping on a treadmill

with body weight support (Dietz et al., 1995, Dobkin et al., 1995, Harkema et al., 1997). Also during assisted locomotion within the driven gait orthosis Lokomat, as done in this thesis, well-modulated locomotor EMG pattern in complete SCI subjects could be induced (Dietz et al., 2002). This rhythmic modulation of locomotor EMG activity is comparable to that of healthy subjects, but the EMG amplitude is strongly reduced.

1.2.3 Plasticity of human spinal locomotor circuitry

Spinal locomotor circuitries are controlled by a variety of input. Spinal reflex (SR) pathways and descending pathways converge on common spinal interneurons (Dietz, 2003, Schomburg et al., 2000). An injury to the spinal cord disrupts the descending pathways and leads to a loss of supraspinal control. Although the spinal neuronal locomotor circuitries underlying the generation of locomotor movement is still intact, the appropriate drive to induce its activity lacks. The induction of a rhythmic locomotor EMG pattern in complete SCI subjects mainly depends on an appropriate afferent input, such as of load and hip joint receptors (Dietz et al., 2002).

Despite the assumption of preserved functional spinal locomotor neuronal hardware, the locomotor EMG pattern induced during assisted locomotion undergoes alterations during the time course of a complete SCI. In chronic SCI subjects a premature exhaustion of locomotor activity, reflected in a drop of leg muscles EMG activity, occurs during a long-lasting session of assisted walking in the driven gait orthosis Lokomat (Dietz and Muller, 2004). This EMG exhaustion phenomenon occurs about one year after a complete SCI, is assumed to take place at interneuronal level and does not depend on the level of lesion. It is assumed that the lacking supraspinal input induces a degradation in spinal neuronal function responsible for the exhaustion phenomenon (Dietz and Muller, 2004). The findings suggest a change of spinal neuronal function below the level of lesion after an SCI. These changes in spinal neuronal circuits are not well understood yet, although the significance is obvious: SCI subjects can only profit of any kind of future regeneration-inducing therapies if the function of spinal neuronal circuitry below the level of lesion is preserved (Curt and Dietz, 2005).

Therefore, the investigation of changes in the function of spinal neuronal circuits is of major interest and a main topic of this thesis. Analysis of locomotor EMG pattern in SCI subjects is just one opportunity to get more insight into spinal locomotor circuitries, whereas investigations of polysynaptic SR associated with locomotor circuitries open another one.

1.3 Spinal reflexes

The flexibility of spinal interneuronal networks is underestimated and SRs are often described as being highly stereotyped. At the beginning of the last century Sherrington (1906) described a high modifiability of SR to the type, the intensity, the site of actions of electrical stimulation and their context.

In general, an SR is an EMG response which appears with a fixed latency in muscles of the same limb and is evoked by a mechanical or electrical stimulation applied to the skin, a muscle or a nerve. The term 'spinal reflex' used in this thesis is defined as a polysynaptic reflex evoked by a non-noxious tibial nerve stimulation. It is assumed that the SR is mainly mediated by cutaneous afferents, in line with reflexes described elsewhere (Duysens et al., 2004). In contrast to a monosynaptic reflex, polysynaptic reflex responses include several interneurons intercalated in the signaling pathways. The reflex responses can consist of an early (latency 60 - 120 ms) and a late (latency 120 - 450 ms) component and appear in synergistic muscle groups - mainly in the ipsilateral leg flexor muscles.

In healthy subjects the SR response evoked by tibial nerve stimulation results in an early SR component, while SR responses after an SCI alter (Hiersemenzel et al., 2000, Hornby et al., 2003).

In acute severe lesion to the spinal cord changes in SR behaviour depend on the time after SCI which can be divided into three phases: spinal shock, transition phase and spastic syndrome where changes in SR behaviour occur (Hiersemenzel et al., 2000). During spinal shock, i.e., in very acute SCI subjects, polysynaptic SRs are lost, while monosynaptic H-reflexes stay stable. Between 2 and 6 months post injury spasticity develops and the early SR component recovers in the time from transition phase to spastic syndrome. The amplitude of the early SR decreases with longer SCI lesion duration and around 5 - 6 months after SCI onset an additional late SR component (latency 120 - 450 ms) appears.

Correspondingly to the SR behaviour in human SCI subjects, SRs are lost in rats after spinal cord transection, but are restored several weeks later (Lavrov et al., 2006, Valero-Cabre et al., 2004). Since in the rat the re-appearance of early SR after SCI is associated with the recovery of locomotor function, we aim to investigate relationship between SR and locomotor behaviour after human SCI.

1.4 Aim of the thesis

As reviewed in the previous sections, the changes in spinal locomotor circuitries after an SCI are not well understood yet. Little is known about the mechanisms underlying the time course of spinal interneuronal activity after SCI and, consequently no countermeasures exist presently to prevent a neuronal degradation of interneuronal function. Preserved spinal neuronal function below the level of lesion will, however, be a prerequisite for the success of regeneration-inducing therapies overcoming the lesion site. Thus, the behaviour and function of interneuronal circuits underlying stepping movements and SR will be explored in human SCI.

The specific issues addressed in this thesis are the following:

- Investigation of changes in spinal neuronal circuits underlying locomotor rhythm generation and SR associated with locomotion during the course of an SCI (possible basis of the degradation of motor function observed in chronic SCI subjects) (study 1,2,3)
- Investigation of the relationship between SR activity and locomotor ability in SCI subjects (study 1,2)
- Development of countermeasures in form of intervening therapies to prevent the development of neuronal dysfunction below the level of lesion (study 2)
- Comparison of changes in spinal neuronal circuits, i.e., neuronal dysfunction, after two forms of central nervous system lesions: SCI versus stroke subjects (study 4)

2 Study 1: Changes in spinal reflex and locomotor activity after a complete spinal cord injury [1]

2.1 Abstract

Locomotor activity and spinal reflexes (SRs) show common features in different mammals, including humans. Here we report the time course of the development of locomotor activity and SRs after a complete spinal cord injury in humans. SRs evoked by tibial nerve stimulation were studied, as was the leg muscle electromyography activity evoked by mechanically assisted locomotion (Lokomat) in biceps femoris, rectus femoris, tibialis anterior and gastrocnemius medialis. Around 8 weeks after the injury, an early SR component (latency 60 - 120 ms) appeared, as in healthy subjects, and a well-organised leg muscle activity was present during assisted locomotion. At around 6 months after injury an additional, late reflex component (latency 120 - 450 ms) appeared, which remained even 15 years after the spinal cord injury. In contrast, the early component had markedly decreased at 18 months after injury. These changes in SR were associated with a loss of electromyography activity and a successively stronger electromyography exhaustion (i.e. decline of electromyography amplitude), when comparing the level of electromyography activity at 2 and 10 min, respectively, during assisted locomotion. These changes in electromyography activity affected mainly the biceps femoris, gastrocnemius medialis and tibialis anterior but less the rectus femoris. When the amplitude relationship of the early to the late SR component was calculated, there was a temporal relationship between the decrease of the early component and increase of the late component and the degree of exhaustion of locomotor activity. In chronic, severely affected but sensori-motor incomplete spinal cord injury subjects a late SR component, associated with an electromyography exhaustion, was present in

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subjects who did not regularly perform assisted stepping movements. Our data are consistent with the proposal of a common mechanism underlying the changes in SR activity and locomotor activity after spinal cord injury. These findings should be taken into consideration in the development of novel rehabilitation schemes and programs to facilitate regeneration-inducing therapies in spinal cord injury subjects.

2.2 Introduction

In subjects with a complete spinal cord injury (SCI), the characteristic pattern of locomotor electromyography (EMG) activity can be induced when the movements are assisted and an appropriate afferent input is provided (Dietz et al., 2002, Harkema et al., 1997). In such a condition the timing of the leg muscle activity is preserved, while the EMG amplitudes are much reduced as compared to walking in healthy subjects. Recently in chronic (> 1 year after injury) complete SCI subjects an exhaustion of locomotor activity, i.e., a decline of EMG amplitude, was reported for assisted stepping (either by the use of a driven gait orthosis (Lokomat) or by therapists), when comparing the level of EMG activity at 10 min versus the onset of a training session (Dietz and Muller, 2004). A possible degradation of spinal neuronal function appeared to occur in chronic complete SCI subjects (Dietz and Muller, 2004). This phenomenon is of functional importance because chronic spinal cord injury subjects can only profit from regeneration-inducing therapies if spinal neuronal function is preserved below the level of lesion (Curt and Dietz, 2005). For the development of appropriate countermeasures to avoid EMG exhaustion, more information about the pathophysiological basis of changes in neuronal function that occur during the course of a complete SCI is required. The progressive changes occurring in spinal reflexes (SRs) after an SCI might provide additional information about the mechanisms underlying the exhaustion. A close relationship between SRs and spinal locomotor activity in cat and rat has been documented elsewhere (Grillner and Shik, 1973, Jankowska et al., 1967, Lavrov et al., 2006, Pierrot-Deseilligny and Burke, 2005). For example, electrical stimulation of high threshold afferents in peripheral nerves can produce an alternating flexor and extensor activation (Grillner, 1969, Grillner and Zangger, 1979). The term ‘spinal reflex’ was chosen here to be defined as a below-nociceptive threshold to tibial nerve stimulation, since a noxious stimulus cannot be determined in complete SCI subjects. The SR evoked in SCI subjects is assumed to correspond to the polysynaptic SR associated with the recovery of locomotor function (Lavrov et al., 2006) and the emergence of spastic

movements that has been observed in rats with a transected spinal cord (Bennett et al., 2004). It is suggested to be mediated by some of the same neurons that make up the locomotor pattern generator (Bussel et al., 1989, Dietz, 2002, Pierrot-Deseilligny and Burke, 2005). Several studies concerning the behaviour of SR in complete SCI have been reported (Hornby et al., 2003, Muller and Dietz, 2006, Schmit et al., 2000). However, no systematic analysis exists on the course of early and late SR components during the course of a complete SCI. The aim of this study was to analyse the relationship between the different components of the SR and locomotor activity during the course of a complete human SCI. We hypothesise that the exhaustion of locomotor activity is associated with changes in the behaviour of SR components.

2.3 Methods

2.3.1 General procedures and subjects

The study protocol was approved by the local Ethics committee and conformed to the Declaration of Helsinki. All participants gave written informed consent before data collection. In addition, to expand the numbers for our analysis, some earlier recordings of SR activity (4 subjects) (Hiersemenzel et al., 2000) and locomotor activity (4 subjects) (Dietz and Muller, 2004) in complete SCI subjects were included. Altogether, 34 subjects with motor complete SCI (AIS A/B) (Maynard et al., 1997) and 5 subjects with sensori-motor incomplete SCI (AIS C) were included in this study. Mean age was 37.4 years (SD = 11.9 years) and neurological level of lesion was between C4 and T11 in subjects with motor complete SCI. In subjects with sensori-motor incomplete SCI, mean age was 46.7 years (SD = 18.3 years) and level of lesion was between C5 and L3. The time span between the SCI and the recordings ranged from 2 months to 15 years in the AIS A/B subjects, and from 2 to 7 years in the AIS C subjects. SRs and leg muscle electromyography (EMG) during assisted locomotion were recorded in 28 SCI subjects and 26 SCI subjects, respectively. Clinical data for all of the SCI subjects and the source of the data are given in Table 2.1. All SCI subjects showed slight to moderate signs of spasticity. About half of the SCI subjects were on anti-spastic medication (usually 20 - 60 mg Baclofen). The time interval between recordings of both locomotor activity and SRs within one subject was at least 1 month. Leg movements of SCI subjects were assisted during locomotion by a driven gait orthosis (DGO) Lokomat (Hocoma AG, Volketswil, Switzerland). The DGO controls the patients' leg trajectories in the sagittal plane during

walking. The hip and knee joints of the DGO were actuated by linear back-drivable actuators integrated into an exoskeleton structure. The legs of the subjects walking in the DGO moved along a pre-defined trajectory. Subjects wore a harness and were fixed to the DGO by straps around their trunk and pelvis. The legs of the device were attached to the subjects' legs with cuffs around the thighs and calves. Proximal and distal leg structures of the DGO were adjusted to align hip and knee joints of the subjects with the joint axes of the DGO. To prevent plantar flexion of the feet, straps were attached around the forefoot and mounted to the DGO. During walking within the DGO, speed was kept constant at 2.0 km/h (0.56 m/s). Cadence had to be slightly adjusted based on leg length of the subjects. A detailed description of the Lokomat is published elsewhere (Colombo et al., 2000, Colombo et al., 2001). Subjects were connected to a body weight support (BWS) system and walked with 65 – 75% BWS. BWS was adjusted in such a way that subjects were loaded with the maximal tolerable body weight without non-physiological knee flexion during the stance phase or toe dragging during swing phase. BSW was not changed during a walking session. Subjects walked for 10 - 15 min within the DGO.

Subject	*Age*	*Gender*	*Level of lesion*	*ASIA*	*Duration of lesion (months)*	*SR recording*	*Locomotor activity recording*	*Source of data*
S1	29.6	m	T4	A	23	+	+	1
S2	38.6	m	T5	A	130	+		1*
S3	56.7	m	C7	A	178	+		1
S4	26.6	f	T5	A	74	+	+	1
S5	57.7	m	T4	B	41	+	+	1*
S6	50.7	m	T11	A	52	+		1
S7	29.8	m	C7	A	109	+	+	1
S8	43.8	m	T7	A	120	+	+	1*
S9	25.8	m	C4	A	62	+	+	1
S10	38.9	m	T9	A	4	+		1
S11	41.9	m	T3	A	6	+	+	1
S12	29.9	m	T10	A	3	+	+	1
S13	45.7	m	T4	A	18	+		1
S14	19.2	m	C5	A	5	+		1
S15	29.7	f	T9	A	131	+	+	1
S16	39.7	m	T5	A	148	+	+	1*
S17	33.6	m	T8	A	85	+		1
S18	40.9	m	T7	A	86	+		1
S19	34.9	m	T5	A	165	+		1
S20	59.4	m	T11	A	2	+		3
S21	28.2	m	C7	B	6	+		3*
S22	49.1	m	T9	A	6	+		3
S23	28.1	m	T5	A	3	+		3*
S24	28.6	m	T10	A	5		+	1
S25	46.5	m	C6	B	32		+	1*
S26	30.0	m	T8	A	5		+	2
S27	32.5	m	T6	A	8		+	2
S28	24.6	f	T1	B	34		+	2
S29	25.0	m	T6	A	12		+	1*
S30	19.7	m	T5	A	5		+	2
S31	43.3	m	T10	A	11		+	1
S32	67.2	m	T5	A	6		+	1
S33	45.1	m	C7	B	5		+	1*
S34	29.5	m	T3	A	3		+	1
S35	34.1	m	L3	C	84	+	+	1
S36	42.6	m	T12	C	67	+	+	1
S37	68.9	m	C5	C	91	+	+	1
S38	33.0	m	C7	C	32	+	+	1
S39	25.7	m	T9	C	29	+	+	1

Tab 2.1: Characteristics of the SCI subjects included in the study.

* = more than one recording.

Abbreviations: C = cervical; T = thoracic; ASIA classification, A = sensorimotor complete, B = motor complete, sensory incomplete; m = male; f = female; SR = spinal reflex. Source of data: (1) new data, re-evaluated data from (2) Dietz and Muller (2004) and (3) Hiersemenzel et al. (2000).

2.3.2 Leg muscle activity during assisted locomotion

Leg muscle activity during assisted walking within the DGO was analysed. Leg muscle EMGs from biceps femoris (BF), rectus femoris (RF), gastrocnemius medialis (GM) and tibialis anterior (TA) from both legs were recorded using surface electrodes (Noraxon, Cologne, Germany). EMG recordings were amplified, filtered (bandpass 30 - 300 Hz) and sampled at 1000 Hz via a 12-bit A/D-converter and stored on a standard PC. An additional trigger signal identifying the heelstrike was recorded. For data analysis and recording the commercial software Soleasy (ALEA Solution GmbH, Zurich, Switzerland) was used. The EMG amplitude of leg muscles during locomotion was analysed using the root mean square (RMS) value per stride. This value represents the mean or effective amplitude per stride (Dietz and Muller, 2004). The resulting data were smoothed using a moving average (window width: 25 strides equivalent to ~1 min of walking) to compensate for stride-to-stride variability. Data were screened for outliers (mean ± 3 SD). Some of the subjects showed some clonus jerks in the GM at the beginning, but these usually disappeared after ~2 min of assisted walking. Therefore, smoothed RMS values of all leg muscles of the first gait cycle after 10 min of walking were normalised to the RMS values of the first gait cycle after 2 min of walking. This should ensure that only the drop of muscle activity occurring during 10 min of assisted locomotion was assessed. These values were also screened for artefacts in signal strength. Quantified EMG values of both legs of one subject were taken together. Some of the SCI subjects were measured at different time points after injury (including those from an earlier study, (Dietz and Muller, 2004), resulting in a total of 36 EMG measurements from 21 motor complete and 5 incomplete SCI subjects during assisted locomotion. All data were recorded under similar conditions.

2.3.3 Spinal reflexes

To assess changes in SRs during the course of an SCI, 23 motor complete and 5 incomplete SCI subjects were examined. Subjects were classified as AIS A, B or C (Maynard et al., 1997) and all showed signs of spasticity and exaggerated reflexes. As a

control group, 10 healthy subjects (mean age: 38.2 years; SD: 12.8 years) were recorded under similar conditions. The SR was elicited by electrical stimulation of the distal tibial nerve at the dorsal aspect of the medial malleolus with the electrical stimulator AS 100 constant current source (ALEA Solutions GmbH, Zurich, Switzerland). The electrical stimulus consisted of a train of 8 biphasic rectangular pulses with single stimulus duration of 2 ms and a frequency of 200 Hz (Muller and Dietz, 2006). The total stimulation duration amounted to 40 ms. The stimulation intensity used was set at two times motor threshold (first visible contraction of the abductor hallucis muscle) (Hiersemenzel et al., 2000). In the healthy subjects, this stimulation strength was experienced as non-noxious. During the measurements subjects were in an upright position, fixed within a harness and completely unloaded from their body weight. Stimulation was elicited 10 times and randomly released every 30 – 45 sec to minimise habituation (Fuhrer, 1976, Shahani and Young, 1971). The SR recordings were performed in all subjects before, and in two subjects also after, the locomotion session.

In some of the SCI subjects listed in Table 2.1, SR recordings from an acute state (within 3 - 6 months after injury) were included in an earlier study (Hiersemenzel et al., 2000) using a similar stimulation approach (stimulation frequency of 100 Hz). The measurements were performed in a recumbent position. The SR showed no difference between an upright and supine (Hiersemenzel et al., 2000) body position.

SR activity was analysed only in the TA muscle of the stimulated leg, as in earlier recordings (Muller and Dietz, 2006) reflex responses could only rarely be separated in the BF. SR responses were analysed for presence of early and late components separately. Time windows were set from 60 to 120 ms for the early component and from 120 to 450 ms for the late component after stimulation onset. The presence of reflex responses within these time windows was determined by an increase of EMG activity three times above standard deviation from the mean baseline. If a reflex response was detected, the highest peak amplitude within the corresponding time window was determined, and the RMS value of 25 ms before and 25 ms after the peak amplitude was calculated. If no response was detectable the value was set to zero. Because of the large inter-individual variability of the reflex amplitudes, we additionally calculated the relationship between the two components over the time after injury. For this, the two reflex components were compared with each other and the greater component was set to 1 and was used for normalisation of the other component. This procedure was chosen because the amplitude relationship between the early and late SR components within a

subject was the focus of interest. The normalised values of each subject were averaged and subsequently the difference between the early and late SR component was calculated to assess the relationship between the two responses.

2.3.4 Statistics

The course of the leg muscle activity during assisted locomotion early and late (chronic) after SCI was calculated. Correlation between exhaustion and duration of lesion was tested using the Spearman rank correlation test. Significance level was set at $p < 0.05$. The correlation between the presence of early and late SR components and the duration of SCI was tested statistically using the Spearman rank correlation test. Significance level was set at $p < 0.05$. The correlation analysis over the course after injury was only performed in the group of motor complete SCI subjects. The course over time of EMG exhaustion and SR components was quantified using a linear regression coefficient. In order to compare the differentially scaled measures, data were z-transformed and the 95% CIs were compared around the regression coefficients.

2.4 Results

In the groups of healthy and SCI subjects the SR was evoked by a similar stimulus intensity at the right tibial nerve (see Methods). In the group of healthy subjects, the tibial nerve stimulation resulted in a single SR response in the TA muscle (mean latency = 78 ms; SD = 12.7 ms). In one subject, inconsistent small late responses were observed (around 170 ms). The stimulus was reported by the subjects to be non-noxious.

In complete SCI subjects, SR could not be evoked during spinal shock and appeared only around 2 months after SCI. Similarly, locomotor activity during assisted locomotion could only be recorded after the period of spinal shock. Except for a higher level of co-contraction of the antagonistic leg muscles and an amplitude reduction of EMG signals in SCI subjects, the locomotor pattern was similar in SCI and healthy subjects. An effect of the anti-spastic medication could not be detected in either the behaviour of SR or in the locomotor activity.

Figure 2.1 shows representative recordings of right leg muscle EMG activity at 2 and 10 min of assisted locomotion in three subjects, in the early (Fig. 2.1A), intermediate (Fig. 2.1B) and later (Fig. 2.1C) stages after a motor complete SCI. After the spinal shock, leg muscle activity re-appeared and increased progressively up to 3 - 4 months after SCI (Fig. 2.1A) while little EMG exhaustion (i.e. decline of EMG amplitude)

occurred during assisted locomotion over 10 - 15 min in BF, GM and RF (except for the TA, see below). Around 6 months after SCI the leg muscle EMG already showed some degree of exhaustion during assisted locomotion over 10 min (Fig. 2.1B). During the later period after the SCI (412 months after SCI), a pronounced degree of EMG exhaustion and loss of EMG activity was present during assisted locomotion (Fig. 2.1C).

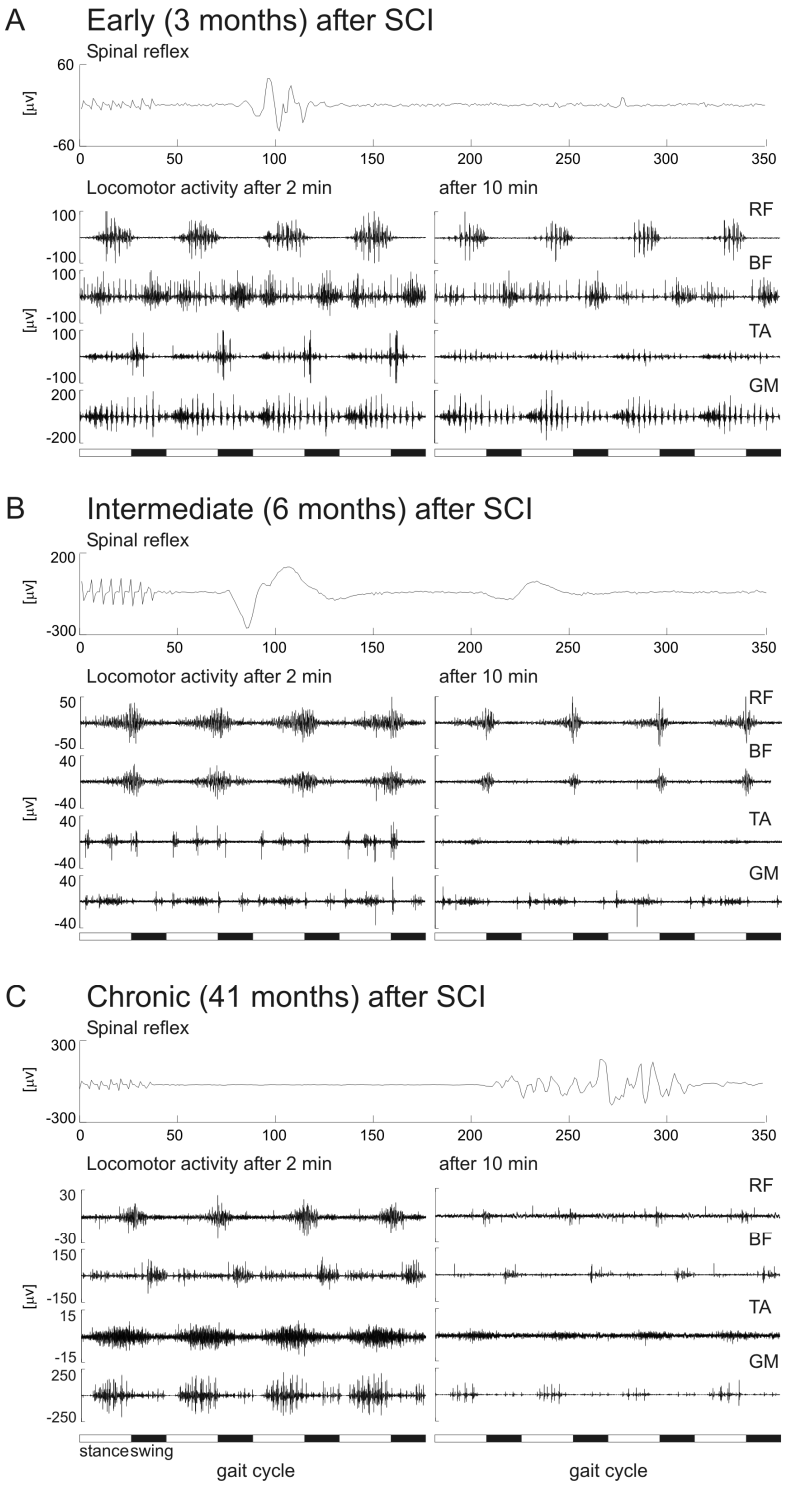


Fig. 2.1: SR and locomotor activity. Representative examples of the two components of SR and locomotor activity at (A) early (S12), (B) intermediate (S11) and (C) late (S5) stages after a complete SCI. The SR was evoked by tibial nerve stimulation (see Methods section) and recorded over the ipsilateral TA muscle. The leg muscle activity is shown at the beginning (left side) and after 10 min (right side) of assisted locomotion.

Figure 2.2A shows the mean values obtained from all subjects with regard to the changes in locomotor activity occurring after a complete SCI. There was a significant degree of exhaustion in BF [Fig. 2.2A(i)] and GM [Fig. 2.2A(ii)], while there was no significant depression of EMG amplitude in the RF [Fig. 2.2A(iii)].

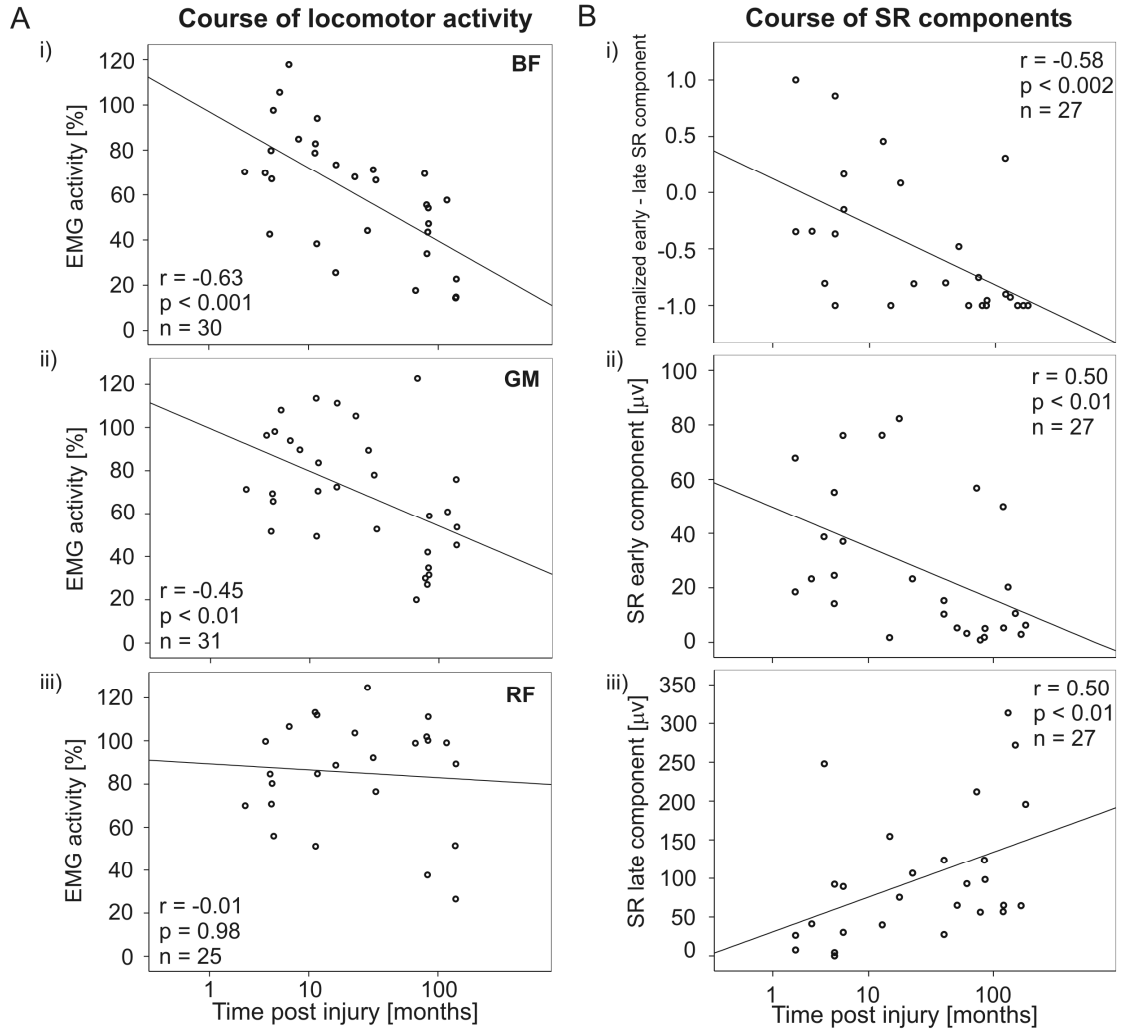


Fig 2.2: Course of locomotor activity and SR. (A) The change in BF (i), GM (ii) and RF (iii) EMG activity during 10 min of assisted locomotion (in percentage of the initial value) and (B) the course of the two SR components (in the TA) is shown for all SCI subjects during the time course after injury (for each graph 25 - 31 measurements). The changes in the SR components are presented as normalised: (i) early minus late component and absolute; (ii) early and (iii) late component values. The time after SCI is displayed on a logarithmic scale.

Figure 2.1 also shows (uppermost records) representative recordings of SR (TA muscle) at early (Fig. 2.1A), intermediate (Fig. 2.1B) and later (Fig. 2.1C) stages after a motor complete SCI. At the early stages after an SCI (Fig. 2.1A: 3 months) only a short latency SR component was present (latency 85 ms). Several months after the SCI (Fig. 2.1B: 6 months) a second late SR component appeared (latency 220 ms), and in the chronic SCI state (Fig. 2.1C: 41 months) only a late SR component (latency 220 ms) was present. There was no difference in the SR behaviour when it was recorded before and after the locomotor session (two complete SCI subjects).

Figure 2.2B shows the mean values obtained from all subjects with regard to the development of early and late SR components. The window for the analysis of the early component was set to 60 - 120 ms and that of the late to 120 - 450 ms (see Methods section). Figure 2.2B(ii) shows that there was a significant decrease ($p < 0.01$) to a very low level after 100 months. The late SR component was more variable and instead increased significantly ($p < 0.01$) from a low level during the first few months (starting around 2 months after SCI) to a higher value after 100 months [Fig. 2.2B(iii)]. Figure 2.2B(i) shows the normalised difference between the early and the late response. Except for the first few months the late SR response dominated as presented here. The characteristics of the SR did not differ between AIS A ($n = 21$) and AIS B ($n = 2$) subjects.

The leg flexor muscle TA showed a high degree of exhaustion already at 3 months (Fig. 2.1A), which became and more pronounced at later stages (Fig. 2.1B and C). Around 1 - 1.5 years after SCI no or little TA EMG activity (55 µV) could be recorded during assisted locomotion in most subjects (Fig. 2.3A and B). However, a late SR component could still be evoked in the TA at this stage. The GM, antagonist of TA at the ankle joint, showed a significant exhaustion [Fig. 2.2A(ii)].

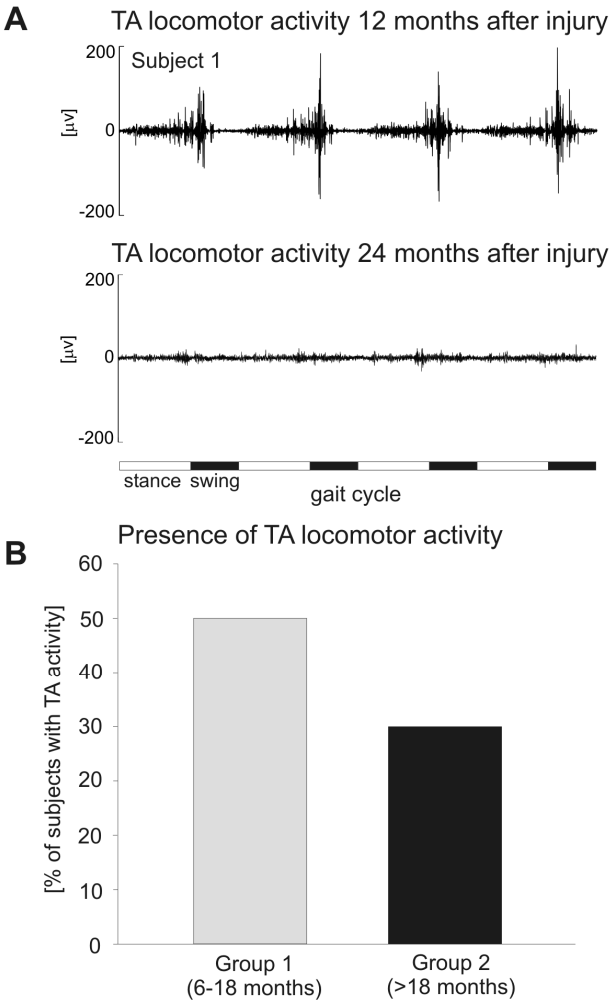


Fig 2.3: Course of TA EMG activity. (A) Example of the TA EMG activity of one subject (S25) at an early stage (12 months) and at a later stage (24 months) after a complete SCI. In contrast to the other leg muscles recorded, the changes in the TA activity consisted mainly in a loss of EMG activity during assisted locomotion. (B) Percentage of subjects displaying the presence of relevant (> 5 µV) TA EMG activity at 6 - 18 months (n = 8) and 18 - 120 months (n = 13) after injury.

In the group of 5 severely affected, but sensori-motor incomplete, chronic (> 1 year) SCI subjects, 3 regularly walked at home with external support (‘therapeutic stepping’), while 2 subjects remained wheelchair-bound. In the walking subjects, the presence of an early SR component was associated with no exhaustion of leg muscle EMG activity during assisted locomotion (Fig. 2.4A). In contrast, in the 2 wheelchair-bound subjects, the presence of only a late SR component was associated with a loss and a pronounced exhaustion of EMG activity after 10 min of assisted stepping (Fig. 2.4B).

There was a similar course of the BF exhaustion and SR components over time: the 95% CI of the regression coefficient calculated for BF exhaustion (-0.357) amounted to (-0.520 to -0.193), and paralleled the course of the SR components [regression coefficient: -0.416; 95% CI: (-0.631 to -0.201)].

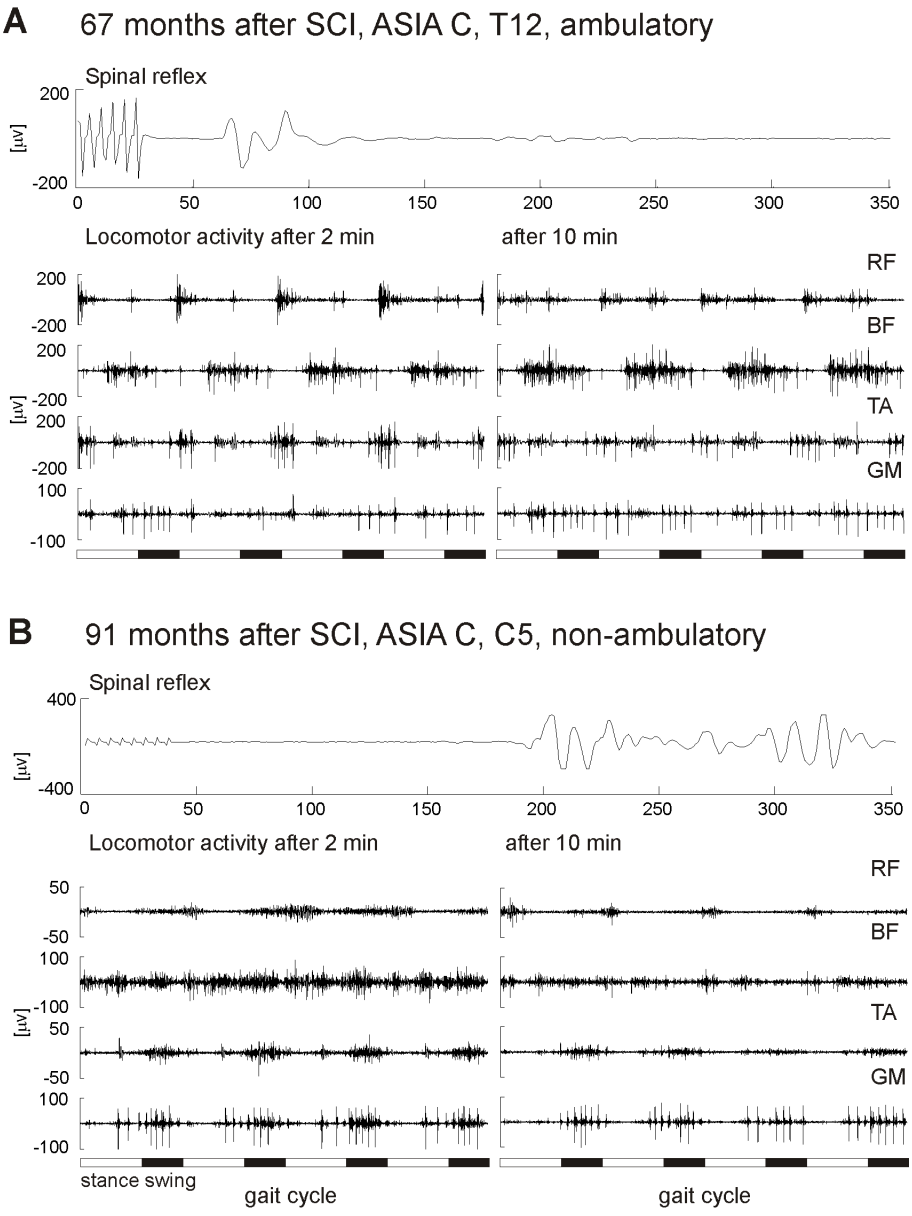


Fig 2.4: SR and locomotor activity in chronic sensori-motor incomplete SCI subjects. Representative examples of SR and locomotor activity in (A) a subject (S36) who regularly walked at home with external support ('therapeutic stepping') and (B) a subject (S37) who remained wheelchair-bound (note the different calibration in A and B).

2.5 Discussion

The aim of this study was to analyse the changes in SR during the course of a complete SCI in relation to changes in locomotor activity. The main observations were:

(i) After recovery from spinal shock, an early SR component and a weak locomotor pattern can be evoked. The pattern becomes strengthened up to 3 - 4 months after SCI. After around 5 - 6 months, both an early and a late SR component can usually be distinguished and the locomotor activity shows some exhaustion towards the end of a 10-min training period;

(ii) After around 1 year, a high level of EMG activity is present initially, but the amplitude of the locomotor activity decreases markedly towards the end of a training session. The exhaustion of the locomotor activity occurs in parallel with an enhanced late and a diminished early SR component;

(iii) From 2 years and up to 15 years after injury the loss of the early component is associated with a prominent exhaustion during a training period and a loss of locomotor EMG activity. The decrease is more pronounced in the BF and TA than in the GM and is almost non-existent in the RF; and

(iv) In severely affected but sensori-motor incomplete, chronic SCI subjects, the early SR component remains preserved and no EMG exhaustion occurs only if they regularly perform stepping movements.

These observations will be discussed below with regard to their patho-physiological relevance and possible therapeutic consequences.

2.5.1 Behaviour of the SRs

Most of the earlier studies on SRs have been performed at different stages of SCI with recordings of either the early or late, or both components of the SR. A great variability has been noted (Andersen et al., 2004, Dimitrijevic and Nathan, 1970). Little attention has been paid to the time course following the SCI with regard to the early and late components of the SR (Hornby et al., 2003, Muller and Dietz, 2006, Schmit et al., 2000).

Several SR studies on SCI subjects have been focused on the relationship with muscle spasms. It has been suggested that a 'wind-up' of these reflexes (Hornby et al., 2003) would occur, with a hypersensitivity to input from force-sensitive muscle afferents (Conway and Knikou, 2008, Schmit et al., 2000), or alternatively, that an expansion of the receptive fields would contribute to the spasms (Andersen et al., 2004). This hyperexcitability has been assumed to be due to a lack of descending inhibitory control and/or increased sensitivity of the SR.

Corresponding to human SCI (Hiersemenzel et al., 2000), the polysynaptic SR is lost after a spinal cord transection in the rat, but becomes restored several weeks later (Lavrov et al., 2006, Valero-Cabre et al., 2004). Also the direct leg muscle EMG and the H-reflex responses show a similar course after an acute complete SCI in humans (Hiersemenzel et al., 2000) and rats (Lavrov et al., 2006). Here we found that 6 - 12 months after complete SCI, the early SR component becomes successively smaller, while the late component increases. Between 2 and 15 years after an SCI, the late component was preferentially present. At this stage, an SR could also be recorded in the contralateral GM (Muller and Dietz, 2006). SR did not change within a walking session.

In the spinal cat an early and a late reflex was evoked, mediated through different pathways (Jankowska et al., 1967). A reciprocal relationship of the two reflexes was shown to occur. The early short-latency flexion reflex (central latency 2 - 3 ms) was elicited in the acute spinal state. If L-DOPA was administered (acting through release of noradrenaline in the spinal cord), the early reflex was depressed, while a long-lasting, long-latency reflex discharge was released instead (Jankowska et al., 1967). This long-latency discharge was considered to represent an activation of the spinal locomotor circuitry (Forssberg and Grillner, 1973, Grillner and Zangger, 1979). Although the mode of stimulation was different, it is assumed that this long-latency discharge corresponds to the SR associated with the recovery of locomotor function in the rat with a transected spinal cord (Lavrov et al., 2006) and with the appearance of the early component of the SR described here. For the late component, a chronic SCI animal is not yet available.

Here we have observed first, that the early component of the SR dominates in healthy subjects and during the first months after a complete SCI and second, that during the later course of an SCI the early component becomes successively depressed while the late SR component increases. This was the case under the condition that a similar stimulus intensity was applied in all subjects. By an increase of stimulus intensity an

early SR component might also be evoked in some chronic SCI subjects (Shahani and Young, 1971) that is the change might just represent a threshold effect.

2.5.2 Behaviour of locomotor activity

In humans, around 2 - 3 months after a complete SCI, a locomotor pattern can be recorded with a normal timing but with a strongly reduced EMG amplitude (Dietz, 2002, Dietz and Muller, 2004). On the basis of this low level amplitude, no further relevant drop in EMG amplitude occurred during assisted locomotion.

Around 6 - 12 months after the SCI a progressive drop and a loss of EMG amplitude was observed during the walking episodes. This phenomenon mainly occurred in leg flexor muscles. The EMG exhaustion has been assumed to occur on a pre-motoneuronal level (Dietz and Muller, 2004, Muller and Dietz, 2006). The loss of TA EMG activity has been suggested to be due to a reduced common synaptic drive to motoneurons (Hansen et al., 2005). In addition, axonal changes were assumed to be responsible for the loss of TA contraction to nerve stimulation below the level of an SCI (Lin et al., 2007).

It is important to emphasise that an appropriate locomotor pattern could be elicited during the first minutes of a walking episode, even in chronic SCI subjects. However, the motor pattern deteriorated during the 10-min walking period in chronic motor complete SCI subjects to an extent which was neither seen in these subjects early after injury nor in ambulatory ASIA C subjects during assisted locomotion. Thus the appropriate motor program is available initially and can be generated without exhaustion only in the early period after a complete SCI. The reason for the exhaustion remains unclear. It can possibly be ascribed to a synaptic fatigue within the spinal locomotor network and be due to disuse as a result of the motor complete SCI. In such a condition, leg extensors, in contrast to the leg flexors, become continuously more activated by proprioceptive input evoked by external stimuli, i.e., are less deprived from afferent input.

2.5.3 Relationship between SR and locomotor activity

A close relationship between polysynaptic SRs and the generation of a locomotor pattern has been emphasised for both rats (Schouenborg, 2002) and cats (Jankowska and Riddell, 1995). The modulation of SR was shown to be related to specific phases of the step cycle in normal rats and cats and when the spinal cord has been transected (Frigon and Rossignol, 2008, Gerasimenko et al., 2006, Rossignol et al., 2008). Group II and III muscle afferents and cutaneous afferents were shown to be responsible for the

SR responses (Gerasimenko et al., 2006, Jankowska et al., 1967, Lavrov et al., 2006). Consequently, the neurons responsible for the SR in SCI subjects were suggested to be part of the spinal networks generating locomotion, i.e., to share the same spinal circuitry (Bussel et al., 1989, Nicol et al., 1995, Parise et al., 1997).

SRs were suggested to provide a measure for the restoration of spinal neuronal circuits responsible for locomotion after transection of the spinal cord in rats (Lavrov et al., 2006). Correspondingly, in humans early after a motor complete SCI as well as in chronic ambulatory incomplete SCI subjects, the presence of an early SR component was associated with a preserved locomotor activity, as is the case in healthy subjects. Vice versa, the presence of an EMG exhaustion was associated with the dominance of a late SR component. Despite the pronounced exhaustion and loss of TA EMG in chronic complete SCI subjects, an SR could always be evoked in the TA (Muller and Dietz, 2006). The observations made in chronic incomplete (AIS C) SCI subjects suggest that the presence of an early SR component, associated with no EMG exhaustion, depended more on the regular performance of stepping movements than on the 'incompleteness' of the SCI.

Although there is ample evidence for a temporal relationship of the changes in SR behaviour and locomotor activity over time this is not conclusive proof that they necessarily depend on the same underlying mechanisms.

It will be important to further analyse if changes in the locomotor training methods used for SCI subjects will reduce the exhaustion of the locomotor pattern and if this is associated with a persistence of the early SR component. This would represent the prerequisite for a successful regeneration-inducing therapy. It is important to note that the appropriate pattern is available initially during a walking period and that the locomotor network can thus operate, although it has become more fragile.

3 Study 2: Spinal reflex activity: a marker for neuronal functionality after spinal cord injury [2]

3.1 Abstract

Background: Alterations in the function of spinal neuronal circuits underlying locomotion after a spinal cord injury (SCI) are associated with changes in the behaviour of spinal reflexes (SR) in both rats and humans. In healthy subjects the SR consists of a dominant early reflex component, while in chronic, severely affected SCI subjects a later component dominates.

Objective: The aim of this study was to investigate the relationship between SR behaviour and walking ability in para-/tetraplegic subjects.

Method: The SR was evoked by non-noxious tibial nerve stimulation. Walking ability was assessed by functional tests and questionnaires.

Results: There was a correlation between walking ability and SR behaviour in chronic SCI: severely affected SCI subjects unable to walk showed dominant late SR components, whilst in ambulatory SCI subjects an early SR component dominated. A functional training with an improvement of locomotor ability was accompanied by both a shift from a dominant to a smaller late and the appearance of an early SR component.

Conclusions: Our findings indicate that SR can serve as a marker for the locomotor ability of SCI subjects. Neuronal plasticity exploited by a functional training is reflected in both an improvement of locomotor ability and a change in balance of SR components towards the early SR component.

[2] This manuscript is in press in the journal Neurorehabilitation and Neural Repair; Spinal reflex activity: a marker for neuronal functionality after spinal cord injury. The authors were Michèle Hubli, Volker Dietz and Marc Bolliger. Data was assessed and analysed by Michèle Hubli. The manuscript was written by Michèle Hubli and revised by the co-authors.

3.2 Introduction

Spinal polysynaptic reflex recordings in mammals have been used to identify interneuronal circuits that are involved in spinal locomotion (Jankowska, 2001, Kiehn, 2006). There is evidence for a relationship between the time course of these spinal cord reflexes (SR) and locomotor capacity after a spinal cord injury (SCI) in rats (Lavrov et al., 2006) and humans (Dietz et al., 2009).

A severe complete SCI leads to a dysfunction of spinal neuronal circuits. This dysfunction is reflected in the exhaustion of locomotor EMG activity during assisted locomotion and is associated with a change from a dominant early to a dominant late SR component (Dietz, 2010, Dietz et al., 2009, Hubli et al., 2011a). However, subjects with sensorimotor incomplete SCI, who regularly perform stepping movements, show no EMG exhaustion and the early SR component dominates.

The focus of this study was to investigate whether SR behaviour can be used as a marker for the functional state of spinal locomotor circuitries underlying walking ability in SCI subjects. In addition, we evaluated the plasticity of neuronal circuits and investigated whether an improvement in locomotor ability following functional training is accompanied by a shift from a late to an early SR component.

The SR was evoked by cutaneous afferents (Duysens et al., 2004). Since a noxious stimulus cannot be determined in SCI subjects, the term ‘spinal reflex’ was chosen as a response perceived by healthy subjects to be below the nociceptive stimulation threshold. Although we use the term ‘spinal reflex’, we cannot exclude that latter parts of the SR are mediated by supraspinal pathways.

3.3 Methods

3.3.1 General procedures and subjects

The study protocol was approved by the local ethics committee and all patients gave informed, written consent before data recordings. Altogether, SR, walking ability and muscle strength were assessed in 28 subjects with chronic (> 1 year post-lesion) SCI. Ten subjects suffered a motor complete SCI (AIS A/B) (Marino et al., 2003) and 18 subjects a motor incomplete SCI (AIS C/D). The mean age of the 28 subjects was 44.8 years (SD = 14.6 years) and the neurological level of lesion ranged from C4 to L3. All SCI subjects showed slightly increased muscle tone and normal tendon reflexes, i.e., preserved lower motoneuron function. The lesion duration ranged from 1.1 to 37.4 years (10 ± 9.3 years). A total of 12 SCI subjects had tetraplegia, 16 had paraplegia and all showed signs of spasticity. The clinical data of all subjects are given in Table 3.1.

Walking ability was evaluated using three functional tests and one questionnaire. The Walking Index for Spinal Cord Injury II (WISCI II) was used to determine subjects' ambulatory capacity (Ditunno et al., 2000). A score of 0 indicates that a subject cannot stand or walk and the highest score, 20, is assigned if a subject can walk more than 10 m without walking aids or assistance.

The second test was the 10 meter walking test (10MWT) which measures the time (in seconds) it takes a subject to walk 10 m. Details about the standardisation have been described previously (van Hedel et al., 2008). The results of the 10MWT were additionally converted into walking speed with a speed of 0 m/s recorded when the WISCI II score was 0 or 1.

Walking ability during daily life can be validly assessed by the Spinal Cord Independence Measure (SCIM II) mobility part (van Hedel and Dietz, 2009). This questionnaire has several items that quantify mobility and in contrast to other walking tests it assesses mobility in the subject's daily environment. Scores of the SCIM II mobility part range from 0 to 40 with higher scores reflecting better daily mobility.

Additionally, we assessed the lower extremity motor scores (LEMS) by manual muscle testing according to the AIS guidelines (Marino et al., 2003). Five key muscles groups in the lower extremities were assessed bilaterally: i.e., hip flexors, knee extensors, ankle dorsiflexors, great toe extensors, and ankle plantarflexors. Each muscle group was

graded between 0 (no muscle contraction) to 5 (able to withstand maximal resistance), with the maximum score being 50 for both legs.

Subject	Age	Gender	Level of lesion	AIS	Lesion duration [years]	SR recordings	Locomotor training
1	43.1	m	C4	D	15.6	+	
2	61.3	m	C6	D	6.4	+	
3	34.1	m	L3	C	7.1	+	
4	59.9	m	C5	C	7.7	+	+
5	25.7	m	T9	C	2.5	+	+
6	42.6	m	T12	D	5.7	+	
7	33.0	m	C7	C	2.8	+	
8	44.2	m	T4	D	21.0	+	
9	21.7	f	T8	C	7.0	+	
10	61.8	m	T5	C	32.0	+	
11	69.9	m	C4	D	1.8	+	
12	56.0	m	C6	D	7.0	+	
13	69.3	m	T8	D	1.1	+	+
14	41.9	m	C6	C	17.5	+	
15	43.6	f	T11	D	37.4	+	+
16	58.3	m	T 4	B	4.5	+	
17	49.9	m	T 5	A	1.2	+	
18	33.7	f	T 9	A	15.0	+	
19	46.3	m	T 4	A	2.6	+	
20	57.4	m	C 7	A	15.9	+	
21	23.6	m	T 4	A	4.3	+	
22	34.3	m	T 7	A	11.0	+	
23	19.4	m	C 6	B	2.4	+	
24	27.9	f	T 4	B	3.5	+	
25	47.4	m	C 7	B	16.9	+	
26	60.4	m	T11	C	3.8	+	+
27	42.0	f	C6	D	15.6	+	+
28	53.3	m	C5	D	2.5	+	+

Tab. 3.1: Characteristics of the SCI subjects included in the study

Abbreviations: m = male; f = female; C = cervical; T = thoracic; AIS classification: A = sensorimotor complete, B = motor complete, C = sensorimotor incomplete with less than 50% of key muscles below the neurological level with muscle grade of less than three (of five), D = sensorimotor incomplete with more than 50% of key muscles below the neurological level with muscle grade three or more.

3.3.2 Spinal reflexes

SR were elicited by electrical stimulation of the distal tibial nerve at the dorsal aspect of the medial malleolus with the electrical stimulator AS 100 constant-current source (ALEA Solution GmbH, Zurich, Switzerland). The stimulus consisted of a train of eight biphasic rectangular pulses with single stimulus duration of 2 ms and a frequency of 200 Hz (cf. Muller and Dietz, 2006), i.e., the total duration of the stimulation was 40 ms. The motor threshold (MT) was determined in a sitting position by a gradual increase of stimulation intensity in steps of 2 mA until the first visible contraction in abductor hallucis muscle was observed. The stimulation intensity was set to two times MT threshold. Electrical stimulation was elicited ten times in both legs in a supine position and was randomly released every 30 - 45 sec to minimise habituation (Fuhrer, 1976, Shahani and Young, 1971).

3.3.3 Locomotor training

Seven motor incomplete (AIS C/D) and two motor complete (AIS A/B) SCI subjects underwent intense locomotor training. They each received individually adapted locomotor training over four weeks, three to four times a week. One training session lasted 30 to 45 min using either the driven gait orthosis (DGO) Lokomat (Colombo et al., 2000) or conventional treadmill training with partial body weight support. Two SCI subjects were differentially trained: First, in the most impaired incomplete SCI subject, assisted treadmill training was combined with functional electrical stimulation (FES) of the lower limbs, stimulating the tibialis anterior (TA) and peroneus muscles as well as the peroneal nerve behind the caput fibulae. This approach facilitates the swing phase by increasing foot clearance (Popovic and Keller, 2005). Stimuli were manually triggered at the end of every stance phase in both legs to enhance hip, knee and ankle flexion during the swing phase. Second, one motor complete SCI subject (AIS B) was trained using the Lokomat over several years once a week.

SR and functional tests were assessed at the beginning and end of the training period and also six months after the end of training. One incomplete SCI subject did not participate in the follow-up assessments. In the most severely affected, incomplete SCI subject the EMG activity of rectus femoris (RF), biceps femoris (BF), TA and gastrocnemius medialis (GM) muscle was assessed during 15 min of assisted walking in the DGO, to detect changes in EMG amplitudes (Dietz and Muller, 2004), before and after one month of locomotor training.

3.3.4 Data analysis

In correspondence with earlier recordings (Muller and Dietz, 2006), only SR in the ipsilateral TA muscle were analysed. The early and late components of the SR response were analysed separately (Dietz et al., 2009). Time windows were set from 60 to 120 ms after stimulation onset for the early and from 120 to 450 ms after stimulation onset for the late component. The presence of reflex responses within these time windows was determined by an increase of EMG activity three times above standard deviation of the mean baseline activity for at least five samples. If a reflex response was detected, the highest peak amplitude within the corresponding time window was determined and the root mean square (RMS) value of 25 ms before and 25 ms after the peak amplitude was calculated. If no response was detected the value was set to zero.

Due to large inter-individual variability in reflex amplitudes, the relationship between the two components was calculated. The amplitude relationship between the early and late SR component within a subject was the focus of interest. Therefore, the two reflex components were compared and the greater component was set to 1 and then subsequently used for normalisation of the other component. The normalised values of each subject were averaged and thereafter the difference between the normalised early and late SR component was calculated. This difference is used here as the term “SR behaviour”. For further analyses only the normalised values of the functionally worse leg (lower ASIA motor score) were used.

3.3.5 Statistics

Correlations between the functional tests and the SR behaviour were statistically tested using the Spearman rank correlation test. Correlation coefficients between 0.5 and 0.75 and with the number of observations n = 28 indicate moderate to good correlations and correlation coefficients higher than 0.75 indicate very good correlations (Dawson-Saunders B, 1994). Differences in SR behaviour and functional outcome measures before, after and at the 6 month follow-up following locomotor training were tested using the Friedman and Wilcoxon signed-rank test. The significance level for all tests was set at $p < 0.05$.

3.4 Results

As expected, the results of the walking tests varied among the 28 SCI subjects investigated: the SCIM II mobility score varied between 15 - 40 (median, 20.5); the WISCI II score varied between 0 - 20 (median, 12); the gait speed of the SCI subjects varied between 0.0 - 2.95 m/s (median, 0.21 m/s) and the LEMS varied between 0 - 49 (median, 24).

3.4.1 Walking ability and SR behaviour

Figure 3.1 shows the SR behaviour in relation to the three walking tests and questionnaires from all SCI subjects. There was a moderate to good significant correlation (Spearman correlation coefficient; rho = 0.626) between SR behaviour and SCIM II mobility score (Fig. 3.1A). This positive correlation coefficient indicates that the more dominant the early SR component in TA muscle, the higher the independence in daily mobility of the SCI subject (high SCIM II mobility score). Moderate to good correlations were found between the SR behaviour and the WISCI II score (Fig. 3.1B; rho = 0.703) and very good correlations were found for the gait speed (Fig. 3.1C; rho = 0.772). These positive correlation coefficients indicate that the more dominant the early SR component, the less the SCI subjects required assistive devices to walk 10 m (Fig. 4.1B) and the higher was the subject's gait speed (Fig. 3.1C).

The correlations in Fig. 1A and 1C seems to be split up into two clusters of incomplete SCI subjects consisting of 8 subjects showing dominant late SR components (SR behaviour < -0.5) associated with no or poor walking ability and 10 SCI subjects with dominant early SR components (SR behaviour > 0.0) associated with moderate to good walking ability. In addition, one might argue that the high correlation between the SR behaviour and the WISCI II score could be solely attributed to the SCI subjects who were not able to walk (WISCI II score of 0). However, if these subjects were excluded from data analysis, the correlation was still good (Fig. 3.1B; rho = 0.622). There was a moderate to good correlation between SR behaviour and LEMS (rho = 0.611, $p < 0.01$) indicating that greater muscle strength in muscles of the lower extremity is associated with a dominant early SR component.

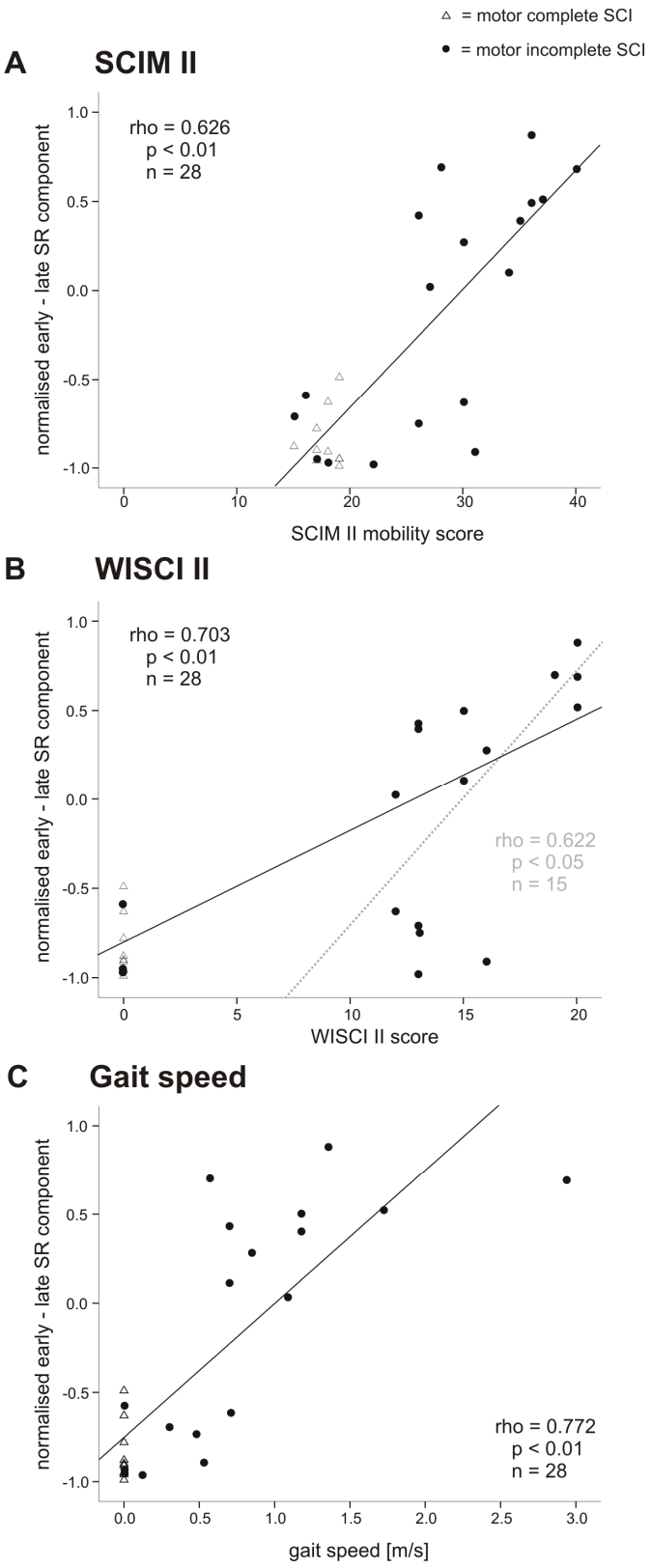


Fig. 3.1: SR behaviour and walking ability. Mean values of all SCI subjects are displayed.

Correlation of the SR behaviour (normalised early - late SR component) and (A) the SCIM II mobility score, (B) the WISCI II score and (C) the gait speed. SCI subjects were divided into two groups according to the completeness of injury, motor incomplete (AIS C/D) and complete SCI (AIS A/B) subjects. A second trend line (B, gray interrupted line) was added to show the correlation when the subjects with a WISCI II score of 0 were excluded.

3.4.2 Effect of locomotor training

Seven incomplete SCI subjects underwent individually adapted locomotor training lasting four weeks. Figure 3.2 shows the changes in SR behaviour and SCIM II mobility score from pre-training, post-training and follow-up assessment for all 7 SCI subjects. In every assessment, stimulation intensity for SR was kept constant at two times the MT of the abductor hallucis muscle. There were improvements in both the SR behaviour (P = 0.018) and the SCIM II mobility score (P = 0.038) after the locomotor training sessions. However, neither of the other walking tests (WISCI II, 10MWT) or the LEMS changed significantly over the four weeks of training or at the follow-up assessment.

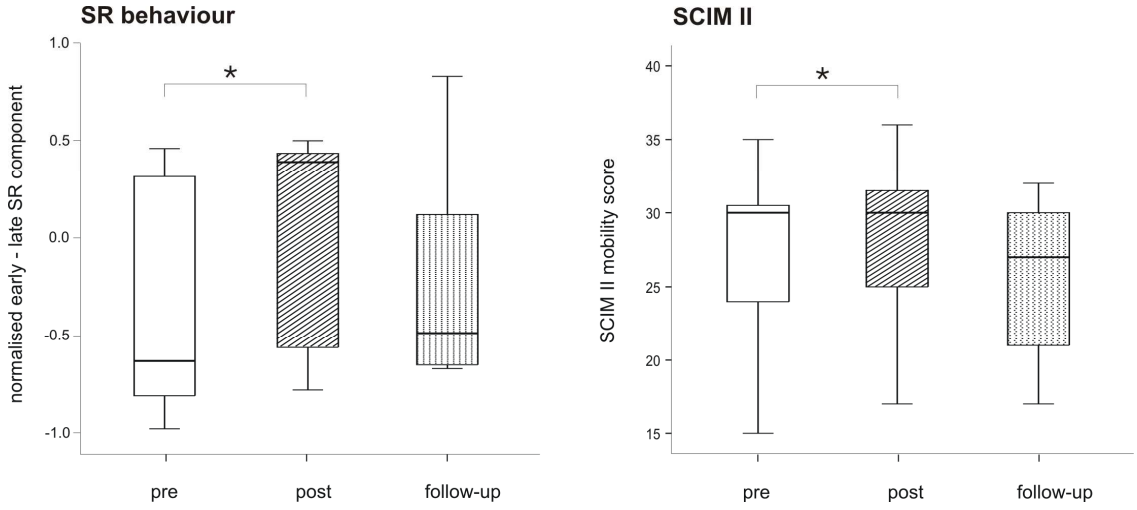


Fig. 3.2: Influence of locomotor training on SR behaviour and SCIM II score. The descriptive statistic of the SR behaviour and the SCIM II mobility score is depicted in boxplots for the pre-training, post-training and follow-up assessments for all trained, incomplete SCI subjects (n = 7, n = 6 for follow-up). Asterisks above the columns define the level of significance of the between-assessments comparison (*, $p < 0.05$).

Figure 3.3 shows the effect of a locomotor training on SR behaviour and functional outcome in a severely affected AIS C subject. The mean values of 10 evoked SR at the beginning (pre-) of, after (post-) training as well as six months after the last training session (follow-up) of this severely affected, incomplete SCI subject are displayed in Figure 3.3A. A late SR component dominated in the pre-training assessment in both TA muscles (latency left 201 ms, right 165 ms). After four weeks of an assisted, body weight supported, treadmill training combined with FES of TA and peronaeus muscles and stimulation of a flexion reflex by strong stimuli applied to the peroneal nerve behind the caput fibulae, an early SR component (latency left 90 ms, right 92 ms) appeared in addition to the late SR component. Six months after the last training session and without stepping practice no early SR component could be elicited (only a late component: latency left 196 ms, right 226 ms). Figure 3.3B shows the functional changes in walking ability of the same subject. The SCIM II mobility score, the 10MWT and the LEMS improved after four weeks of locomotor training but worsened after six months of no locomotor practice at home. In particular the proximal leg muscles (hip flexors and knee extensors) benefited from the locomotor training.

Figure 3.4 shows the changes in leg muscle EMG activity in this severely affected incomplete SCI subject before and after locomotor training. The EMG recordings during assisted walking within the DGO before (Fig. 3.4A) and after one month of a locomotor training (Fig. 3.4B) are shown. At the beginning of the training period the leg muscle activity considerably decreased during 10 min of assisted walking (RF (-67%), BF (-80%), TA (-12%) and GM (-84%)). After the training sessions (Fig. 3.4B) EMG activity showed less of a decrease (RF (-21%), BF (+10%), TA (+8%) and GM (+2%)).

Two motor complete paraplegic subjects served as control subjects. Neither the SR nor the functional outcome was affected by the locomotor training. The late SR component as well as the walking ability assessed by the SCIM II mobility score, the WISCI II and the gait speed persisted over the training period whereas the LEMS remained at baseline values.

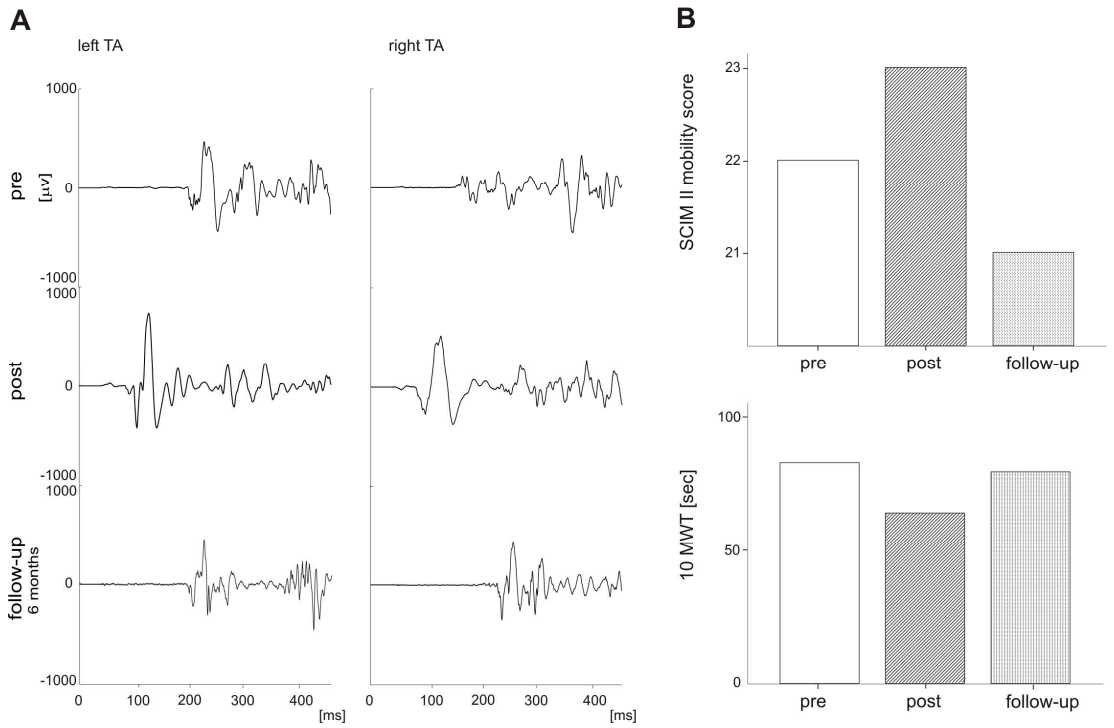


Fig. 3.3: Changes in SR and walking ability after a locomotor training. A representative example of the effect of locomotor training in an incomplete SCI subject (male, 59.9 years; AIS C; level of lesion C5; duration of lesion 7.7 years) is depicted. (A) The SR responses in the TA muscles of the left and the right leg were recorded at three different time points: pre- and post-training and at a follow-up 6 months after the end of the training (training duration was four weeks). (B) Changes in SCIM II mobility score and 10 MWT from pre- to post-training and follow-up assessments.

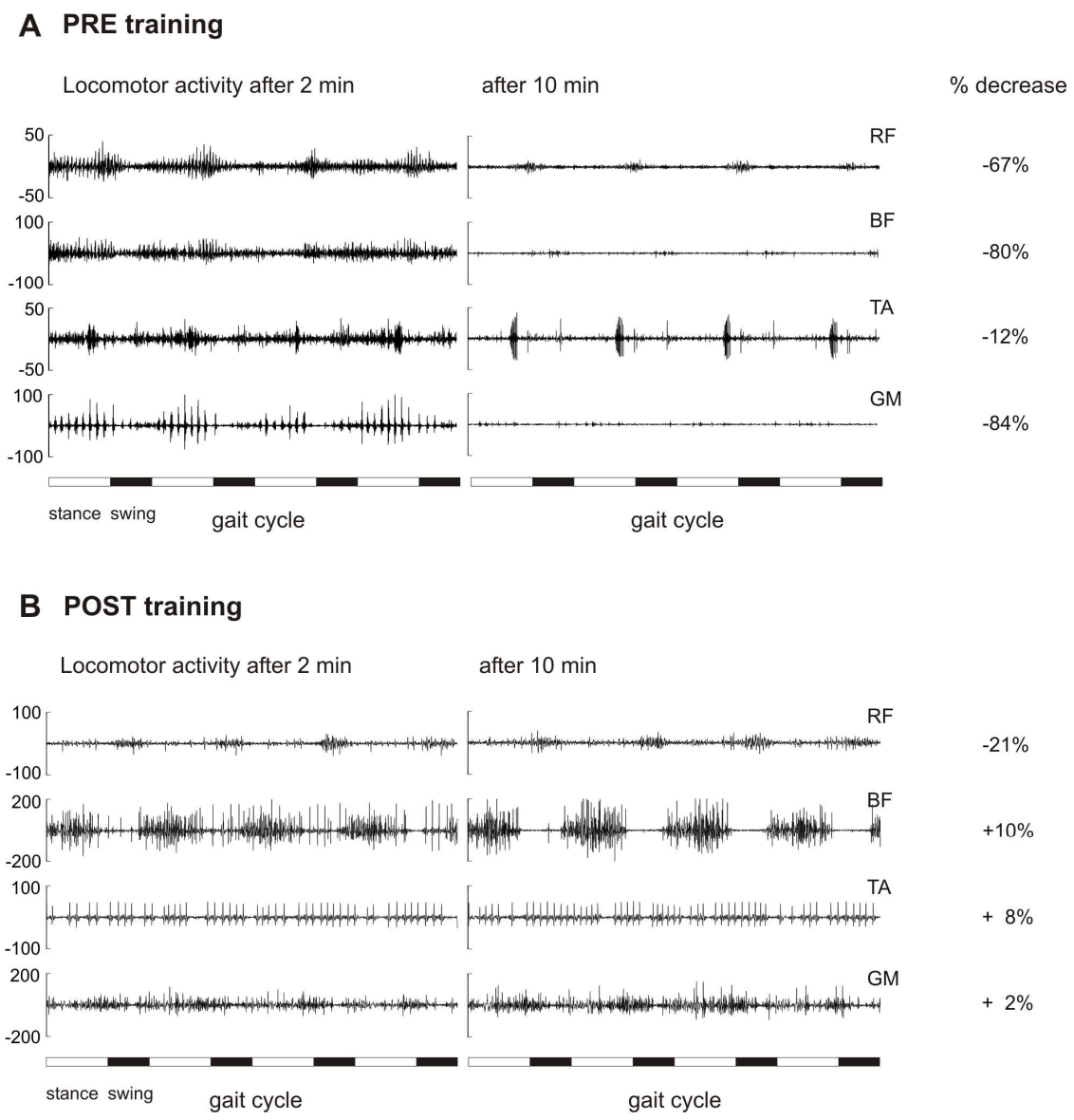


Fig. 3.4: Effect of locomotor training on EMG exhaustion. Representative electromyographic recordings of four leg muscles during assisted walking at 2 and 10 min are displayed for pre- (A) and post-training assessment (B). Relative decreases of RMS value (in % of the initial value) of four gait cycles from 2 to 10 min of assisted walking are depicted on the right line.

3.5 Discussion

This study investigated the relationship between the SR behaviour and walking ability in SCI subjects. The main findings were: (i) the more dominant the early SR component, the better the locomotor ability of an SCI subject. This was reflected in increased daily mobility, less need for assistive walking devices, faster walking speed and higher leg muscle strength. (ii) Intense locomotor training was followed by both a change in balance of SR towards the early SR component and an improvement of walking ability. Thus, SR behaviour might be a useful marker for individual walking ability which is modifiable by locomotor training after an SCI.

3.5.1 SR and walking ability

A close relationship between the generation of SR and locomotor activity has been shown for rats (Lavrov et al., 2006) and cats (Forssberg and Grillner, 1973, Grillner and Zangger, 1979): First, the late SR response in rat with transected spinal cord is associated with the recovery of locomotor function (Lavrov et al., 2006) and this is assumed to correspond to the appearance of the early SR component described here. Second, the long-latency reflex discharges in the spinal cat evoked by administration of $_{L}$-DOPA (Jankowska et al., 1967) was considered to represent an activation of spinal locomotor circuitries (Forssberg and Grillner, 1973, Grillner and Zangger, 1979) and therefore seems to be reflected in the early SR component described here. The comparisons between SR behaviour in human and animal models were made on a functional point of view, although the mode of stimulation was different. In addition, the classical cat model of Jankowska (Jankowska et al., 1967) might not fully fit with the SCI subjects studied here. For example, intrathecal application of clonidine had a strong inhibitory effect on spinal neuronal activity (Dietz et al., 1995) while it enhanced locomotor activity in spinal cat (Barbeau et al., 1987, Forssberg and Grillner, 1973).
Correspondingly in humans the neuronal circuits underlying the SR have been suggested to share the same spinal circuitries for locomotion (Bolliger et al., 2010, Bussel et al., 1989). In chronic non-ambulatory SCI subjects locomotor EMG exhaustion during assisted walking was associated with a shift from an early to a late SR component, while in incomplete SCI subjects the presence of an early SR component was associated with a preserved and stable locomotor EMG activity during 10 min of assisted walking (Dietz et al., 2009).

Our present findings suggest that in chronic, incomplete SCI subjects, the presence of an early SR component is associated with walking ability whereas, in chronic, severely affected SCI subjects an immobility leads to a dominance of the late SR component. Hence the SR component might serve as a marker to identify chronic SCI subjects with 'functional' spinal locomotor circuitries that might have the potential to ambulate.

The functional state of spinal locomotor circuitries, reflected in the SR behaviour, seems to depend rather on the regular practice of locomotor movements in chronic SCI subjects than on the 'completeness' of the SCI. Thus, an appropriate afferent input is essential to maintain spinal locomotor circuitries (Dietz et al., 2002). The loss of this input, due to immobility after a SCI, leads to a neuronal dysfunction of spinal locomotor circuits, specifically comprising a dominant late SR component and EMG exhaustion (Dietz, 2010, Hubli et al., 2011a).

3.5.2 Plasticity of locomotor circuits

Neuronal plasticity within the central nervous system can contribute to improved walking ability after SCI and plays an essential role in the recovery of locomotion (Barbeau et al., 2002, van Hedel and Dietz, 2010). Neuroplastic changes can occur at cortical, brainstem and spinal levels. The effectiveness of locomotor training is based on enhancing neuronal plasticity (Dietz and Harkema, 2004) as well as the reorganisation of sensorimotor pathways (Knikou, 2010).

In the present study the 6 incomplete SCI subjects which received gait training without FES showed only mild, while the one SCI subject that received FES in addition to the gait training showed good improvements of walking ability. Nevertheless, a functional improvement after the training period was always associated with a strengthening of the early SR component.

The rather limited effects on locomotor improvements might be explained by the following aspects. Firstly, the training period over 4 weeks might have been too short to induce stronger long-lasting changes in functional outcome. Secondly, the changes induced in spinal locomotor circuitries were less reflected in the functional outcome measures used and thirdly, 4 (AIS D) out of the 7 SCI subjects already had a moderate to good walking ability (floor effect) at the beginning of the study prior to training. However, the training with the DGO Lokomat is known to be more effective in AIS C SCI subjects (Wirz et al., 2005). Correspondingly, the most severely affected incomplete SCI subject with dominant late SR components at the beginning of the intervention profited

the most from a combined locomotor and FES training in terms of neuronal function, locomotor ability and SR behaviour. Such a combined training approach provides more appropriate input to 'non-functional' neuronal circuits which might turn them into a 'functional' state. This is in line with the observation in rats that a combination of locomotor training, epidural stimulation and pharmacological intervention leads to a conversion of spinal circuits from a 'non-functional' into a 'functional' state (Courtine et al., 2009).

Regular training combined with afferent input to spinal neuronal circuits seems to prevent neuronal dysfunction and is even able to regain normal neuronal function in severely affected, incomplete SCI subjects. In contrast, no change in locomotor capacity occurs in motor complete SCI subjects after intense locomotor training (Wirz et al., 2005). Correspondingly, a dominant late SR component as a marker of no walking ability persisted even after the training in motor complete paraplegic subjects.

This study shows that the SR behaviour changes in parallel with an ambulatory improvement following locomotor training. The effects on SR behaviour can be due to activation of spinal neuronal circuits or, alternatively, to an influence of the training on supraspinal centres. For example the application of FES has been shown to increase the excitability of the cortex or/and corticospinal pathways (Everaert et al., 2010, Thompson et al., 2006).

3.5.3 Physiological considerations

In vertebrates, an interaction between excitatory and inhibitory neuronal circuits shapes the locomotor pattern (Grillner et al., 1995). Following SCI in cats an imbalance between excitatory and inhibitory inputs to the spinal neuronal circuits occurs (Ichiyama et al., 2006, Tillakaratne et al., 2002). As a consequence, blocking inhibitory transmission can markedly improve walking capacity (de Leon et al., 1999). In immobilised SCI subjects inhibitory circuits might become dominant, while the function of excitatory interneurons weakens (Dietz, 2010, Hubli et al., 2011a). As a consequence, there was an exhaustion seen in the EMG amplitude during assisted walking, associated with a suppression of the early SR component and a facilitation of pathways mediating the late SR component. In contrast, in motor incomplete, mobile SCI subjects proprioceptive feedback information drives excitatory spinal neuronal circuits reflected in a dominance of the early SR component.

3.6 Conclusions

Here we show that the SR could serve as a marker for the functional state of spinal locomotor circuitries and could be used as a new tool to assess changes within these circuits. The SR might be used in addition to clinical measures in SCI subjects to estimate the dysfunction of neuronal circuits or the absence of dysfunction, e.g., in psychogenic plegia or plegia due to biomechanical rather than neuronal constraints.

4 Study 3: Influence of spinal reflexes on the locomotor pattern after spinal cord injury [3]

4.1 Abstract

In complete spinal cord injured (cSCI) subjects a shift from dominant early (60 - 120 ms latency) to dominant late (120 - 450 ms latency) spinal reflex (SR) components occurs over time after injury. This shift is assumed to reflect spinal neuronal dysfunction below the level of a spinal lesion. The neuronal pathways of SR are suggested to be closely connected with spinal locomotor circuits. The aim of this study was to explore the interaction of the two SR components with the electromyographic (EMG) pattern induced by assisted locomotion in cSCI subjects. Leg muscle EMG activity was analysed during assisted locomotion in both healthy and motor cSCI subjects. SRs were evoked by non-noxious tibial nerve stimulation during mid-stance phase of the gait cycle. Early and late SR components had a differential interaction with the locomotor pattern. In healthy and cSCI subjects with a dominant early SR component the locomotor EMG pattern was modulated in the form of a short increase in leg flexors activity in the stance phase (tibialis anterior, biceps femoris). In contrast, in chronic cSCI subjects with a dominant late SR component no activation in biceps femoris but a long-lasting activation of tibialis anterior and rectus femoris muscles during the stance phase was evoked. It is concluded that the same tibial nerve stimuli activated two different neuronal pathways, resulting in divergent interactions with spinal locomotor circuitries. It is proposed that the two SR components have different physiological roles during locomotion.

[3] This manuscript is published in the journal Gait and Posture: Interactions of locomotor pattern and spinal reflexes after spinal cord injury. Gait and Posture 34 (2011) 409-14. The authors were Michèle Hubli, Volker Dietz and Marc Bolliger. Data was assessed and analysed by Michèle Hubli. The manuscript was written by Michèle Hubli and revised by the co-authors.

4.2 Introduction

Spinal locomotor networks are capable to produce rhythmic locomotor movements, such as walking, swimming and hopping, independently of sensory afferent input or supraspinal control (Delcomyn, 1980, Grillner et al., 1981, Kiehn, 2006, Rossignol et al., 1996). Several pathways of interneurons have been identified based on reflex circuits that are involved in the generation of the locomotor pattern in mammals (Jankowska, 2001, Kiehn, 2006, McCrea, 2001). The analysis of polysynaptic spinal reflexes (SR) involving those interneuronal circuits can be used as neuronal window into spinal circuits underlying locomotion. Information about the reorganisation of such spinal circuits after a spinal cord injury (SCI) SCI might be provided by the analyses of the reflex behaviour during a locomotor task.

The term 'spinal reflex' is used here to define a response to a probably below-nociceptive threshold stimulation of the distal tibial nerve, since a noxious stimulus threshold can hardly be determined in complete spinal cord injury (cSCI) subjects. We assume that mainly group II afferents were stimulated.

Studies in both animals and humans have been focused on changes in SR behaviour after an SCI. Although different modes of stimulation have been used in animal and human models, the time course of changes in SR behaviour and locomotor activity is to some extent comparable (Dietz et al., 2009, Hiersemenzel et al., 2000, Jankowska et al., 1967, Knikou, 2010, Lavrov et al., 2006). In human cSCI subjects, an absence of SR during spinal shock and the reappearance of an early SR component during the transition phase to spasticity have been described (Hiersemenzel et al., 2000). This has also been shown to occur in the spinal rat using epidural stimulation of lumbar spinal cord (Lavrov et al., 2006). Between 5 and 6 months after injury an additional late SR component appears (Hiersemenzel et al., 2000) and becomes dominant during the further time course while the early SR component decreases (Dietz et al., 2009). The shift from dominant early to dominant late SR component during a cSCI correlates with the degree of exhaustion of leg muscle electromyography (EMG) during assisted locomotion (Dietz et al., 2009, Dietz and Muller, 2004). Hence, it is assumed that SR- and locomotor-generating circuitries share common spinal neuronal pathways. However, it is not yet clear in which way the different reflex pathways of early and late SR components influence spinal locomotor circuitries.

Recently it has been shown that if appropriate proprioceptive input, i.e., during assisted walking, is provided, the early SR component can be regained in some cSCI subjects

(Bolliger et al., 2010). The aim of this study was to investigate the influence of the early and late SR components on the locomotor EMG pattern induced by assisted locomotion in cSCI subjects. We hypothesise that the early and the late SR component, elicited during assisted locomotion, differentially influence with the locomotor EMG pattern. The results might give further information about the changes in spinal neuronal circuits and their function in locomotion generation after a cSCI.

4.3 Methods

4.3.1 Subjects and general procedures

The study protocol has been performed in accordance with the local ethics committee and the Declaration of Helsinki. All volunteers gave written informed consent before data collection. Ten healthy and 20 subjects with motor complete spastic para- or tetraplegia (AIS A/B) (Marino et al., 2003) participated in this study. Clinical data of all cSCI and healthy subjects are given in Table 4.1.

The influence of SR responses on the locomotor EMG pattern was analysed during assisted locomotion on a treadmill within the driven gait orthosis (DGO) 'Lokomat' (Hocoma AG, Volketswil, Switzerland). Subjects were fixed to the DGO by straps around their trunk and pelvis. Furthermore, the legs of the subjects walking in the DGO were fixed with cuffs around the thighs and shanks and were moved along a pre-defined trajectory. Dorsiflexion of the ankles during the swing phase was achieved by passive foot lifters (elastic straps) which prevent stumbling during assisted locomotion of subjects but did not constrict the subjects' range of motion at the ankle. To enable a physiological walking pattern in cSCI, all subjects were unloaded by 70% of their body weight using a harness connected to a body weight support (BWS) system. A more detailed description of the 'Lokomat' can be found elsewhere (Colombo et al., 2001). During assisted locomotion, treadmill speed was kept constant at 2.0 km/h.

Subject	Age (years)	Gender	Level of lesion	AIS	Duration of lesion (months)	Group SR
S1	58.3	m	T4	B	54	2
S2	49.9	m	T5	A	14	1
S3	37.8	f	T8	A	2	1
S4	33.7	f	T9	A	180	2
S5	46.3	m	T4	A	31	1
S6	57.4	m	C7	A	191	2
S7	23.6	m	T4	A	51	1
S8	34.3	m	T7	A	132	1
S9	51.7	m	T11	A	66	1
S10	29.6	m	T10	A	6	1
S11	19.4	m	C6	B	29	1
S12	26.0	m	C4	A	76	1
S13	20.9	m	C5	A	3	1
S14	27.9	f	T4	A	41	2
S15	30.9	m	C7	A	124	1
S16	47.4	m	C7	B	203	1
S17	50.2	m	C7	B	78	1
S18	38.4	m	T8	A	144	1
S19	39.7	m	T5	A	154	2
S20	47.2	m	C5	B	160	1
H1	27.3	m				1
H2	25.1	m				1
H3	54.2	m				1
H4	32.4	m				1
H5	24.3	m				1
H6	35.9	m				1
H7	29.6	m				1
H8	39.9	f				1
H9	24.0	f				1
H10	29.8	m				1

Tab. 4.1: Characteristics of SCI (S) and healthy (H) subjects included in the study. Abbreviations: C = cervical, T = thoracic; AIS classification: A = sensorimotor complete, B = motor complete, sensory incomplete; m = male; f = female; group SR: 1 = dominant early SR component, 2 = dominant late SR component.

4.3.2 Spinal reflexes during assisted walking

SRs were elicited by electrical stimulation of the distal tibial nerve with the electrical stimulator AS 100 constant-current source (ALEA Solution GmbH, Zurich, Switzerland). Bipolar surface stimulation electrodes (Sensor 700, 15x20 mm, Ambu, Denmark) were placed on the left foot at the dorsal aspect of the medial malleolus. The stimulus consisted of a train of 8 biphasic rectangular pulses with a single stimulus duration of 2 ms and a frequency of 200 Hz (cf. Muller and Dietz, 2006) resulting in a stimulation duration of 40 ms. SR threshold was determined in a sitting position by a gradual increase of stimulation intensity with 2 mA steps. In healthy subjects stimulation intensity was risen till the first movement of the big toe (motor threshold) and then doubled for SR elicitation during assisted walking (range 8 - 18 mA). In SCI subjects the stimulation intensity had to be higher for stable SR elicitation during assisted locomotion. Therefore, the stimulation intensity was set to two times the reflex threshold, i.e., the first response in EMG of the tibialis anterior muscle (range 10 - 40 mA). Stimulation onset was set at mid-stance phase of the left leg in order to perturb the locomotor pattern. Ten electrical stimulations were randomly applied at the left tibial nerve with an interval of 30 - 45 sec to avoid habituation (Fuhrer, 1976).

EMG recordings were made from bilateral proximal and distal leg muscles, i.e., rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA) and gastrocnemius medialis (GM), using surface electrodes (Noraxon, Cologne, Germany). The EMG signals were amplified, filtered (bandpass 30 – 300 Hz) and sampled at 1000 Hz via a 12-bit A/D-converter and stored on a standard PC. In addition, left and right heel strikes were recorded and analysed as trigger signals for the onset of tibial nerve stimulation. For data acquisition and processing, the commercially available software Soleasy (ALEA Solution GmbH, Zurich, Switzerland) was used.

4.3.3 Data analysis

Group arrangement of the 20 cSCI subjects was done by analysing the SR responses in the TA muscle for the presence of early and late components. Time windows were set from 60 - 120 ms after stimulation onset for the early and from 120 - 450 ms for the late SR component (Muller and Dietz, 2006, Pierrot-Deseilligny and Burke, 2005). SR analyses were done as described earlier (Dietz et al., 2009) and resulted in a score between 1 and -1 for normalised early minus late SR component (SR behaviour). A

value between 0 and 1 was then referred to a dominant early SR response, while a value between 0 and -1 was referred to a dominant late SR response.

The locomotor EMG signals of all recorded muscles were offset corrected, rectified and for graphical data analysis smoothed using a moving average filter (window width 25 samples). EMG values were calculated as the mean of the 10 pre- or stimulated strides and were normalised to the peak value of the stimulated stride.

The mean EMG amplitude of all muscles in the stride before and during the release of the 10 electrical stimuli was analysed using the root mean square (RMS) value from stimulation onset to end stance phase. In order to obtain net effect of SR elicitation, the EMG of the corresponding time window in the non-stimulated steps cycle before the stimulation was subtracted from the EMG of the stimulated steps.

The background EMG activity was calculated by RMS in a time window of 50 ms before the stimulation onset. Background EMG activity of all muscles was compared between the SCI subjects with early and late SR components and correlated to SR amplitudes in TA of all subjects assessed in this study.

4.3.4 Statistics

Statistical calculations were performed using PASW Statistics 17.0 for Windows (SPSS Inc., Illinois, USA). Analysis of group differences in lesion duration, level of lesion and age of cSCI subjects were performed using the Mann-Whitney U test. Differences in RMS amplitude of the net muscle activities between the groups (healthy, early SR and late SR) were tested statistically using the Mann-Whitney U test. Correlations between the early / late SR component in the TA muscle and its background activity were calculated using Spearman correlation coefficient. The differences in background EMG activity between the groups were tested with a Wilcoxon test. The significance level was set at $p < 0.05$.

4.4 Results

SCI subjects were divided into two groups according to their SR behaviour during assisted locomotion (see Table 4.1). Some 15 cSCI subjects showed dominant early SR (SR behaviour > 0) and 5 cSCI subjects showed dominant late SR components (SR behaviour < 0) during assisted locomotion (see Methods). The mean SR behaviour (normalised early - late SR component) of the early SR group was 0.64 (SD = 0.18) and for the late SR group -0.87 (SD= 0.15). The mean SR behaviour of the 10 healthy

subjects was 0.77 (SD = 0.06). The early and the late SR group did not significantly differ in the lesion duration (early group 6.2 ± 5.4 y, late group 10.3 ± 5.9 y; P = 0.168), level of lesion (early group C4 - T11, late group C7 - T9; P = 0.312) and age of subjects (early group 43.4 ± 13.8 y, late group 36.9 ± 11.4 y; P = 0.349).

Figure 4.1 shows the recordings of the raw EMG from four leg muscles of a cSCI subject produced during assisted locomotion in the 'Lokomat'. The timing of the leg muscle activity pattern with a main activation of the extensors during the stance phase and the flexors during the swing phase of gait was preserved. Nevertheless, the amplitudes were much reduced in contrast to healthy subjects.

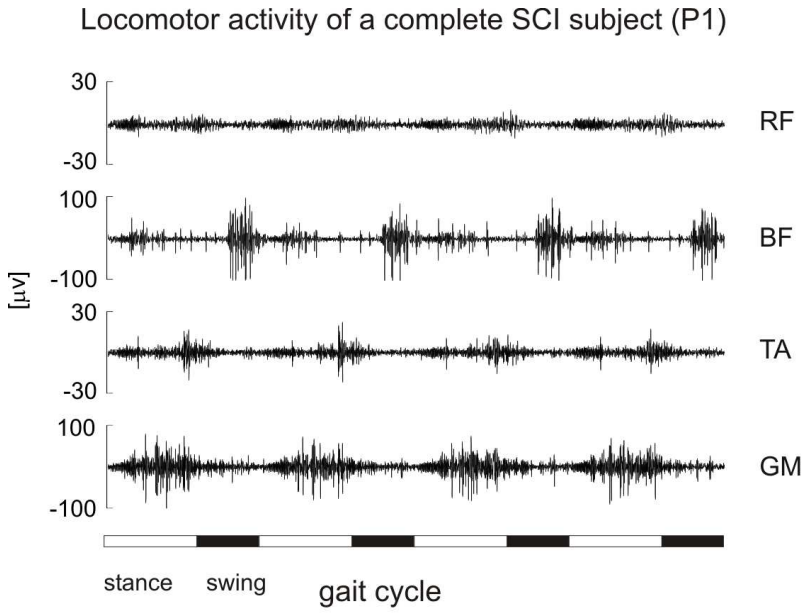


Fig. 4.1: Locomotor activity in a complete SCI subjects during assisted locomotion. A representative example of a raw EMG of four leg muscles in a complete SCI subject is depicted. The subjects walked continuously in the 'Lokomat' (2 km/h; 70% body weight support). The EMG sequences of four steps at 2 min after beginning of assisted locomotion are shown.

Figure 4.2 shows recordings of the locomotor EMG pattern of the left leg muscles during assisted locomotion within the DGO in the non-stimulated and stimulated conditions. The locomotor EMG patterns represent the median value of the following subject groups:

healthy subjects (Fig. 4.2A), cSCI subjects with dominant early SR component (Fig. 4.2B) and cSCI subjects with dominant late SR (Fig. 4.2C).

In healthy subjects, only early SR components with latencies between 60 and 85 ms appeared (mean SR behaviour = 0.77). Reflex responses with such latencies are assumed to be mediated by spinal pathways (involvement of cortical pathways > 120 ms latency). The locomotor EMG pattern was shortly interrupted by the SR responses, consisting of a short TA and BF activation (102 ± 15 ms, width) and a slight inhibition of the ongoing GM activity. After this short interruption the normal locomotor EMG pattern was followed (see Fig. 4.2A). A similar behaviour could be seen in the 15 cSCI subjects with dominant early SR component (see Fig. 4.2B). In contrast, the locomotor EMG pattern of cSCI subjects with dominant late SR component was affected by a long-lasting activation of RF and TA muscles (Fig. 4.2C). This alteration of leg muscle EMG activity lasted over 614 ± 140 ms.

Figure 4.3 shows the mean net influence of early and late SR with leg muscle activity during assisted locomotion, i.e., leg muscle activity of the stimulated steps after subtraction of EMG activity in non-stimulated steps. In healthy subjects, a short increase in leg flexors (TA, BF) and a decrease, i.e., suppression of extensor activity (GM) occurred. In cSCI subjects with a preserved early SR component, a similar modulation of the locomotor EMG pattern was observed, i.e., a short increase in TA and BF activity. In contrast, the net muscle activity modulation by SR differed in cSCI subjects with dominant late SR components: a long-lasting activation, mainly in TA and RF, was present.

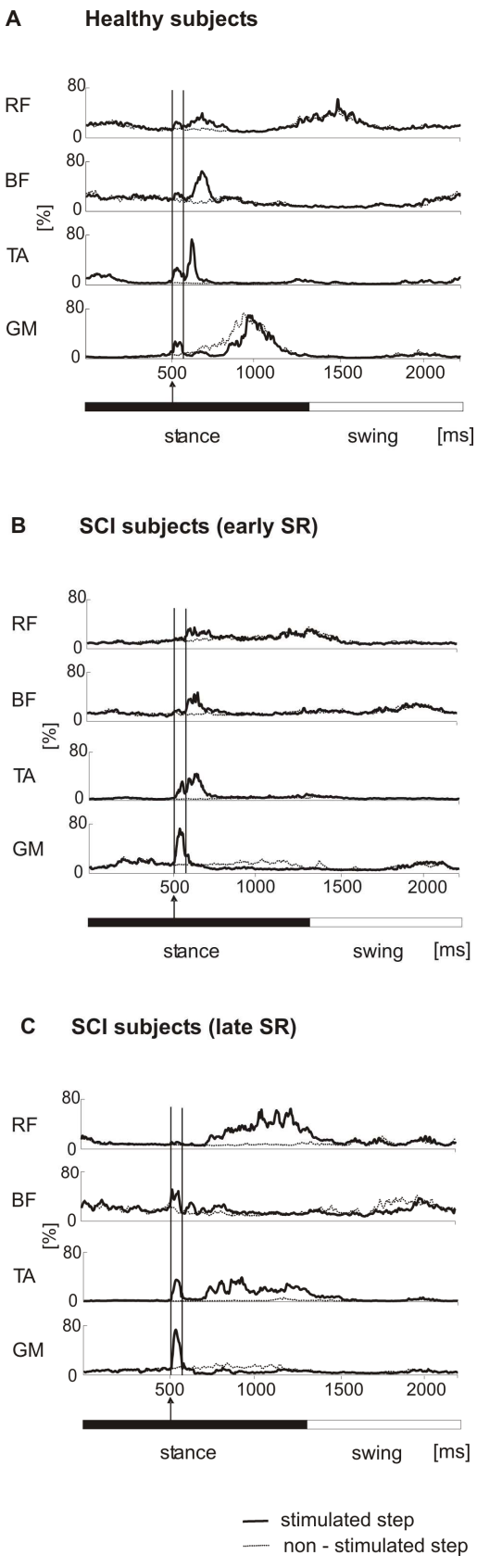
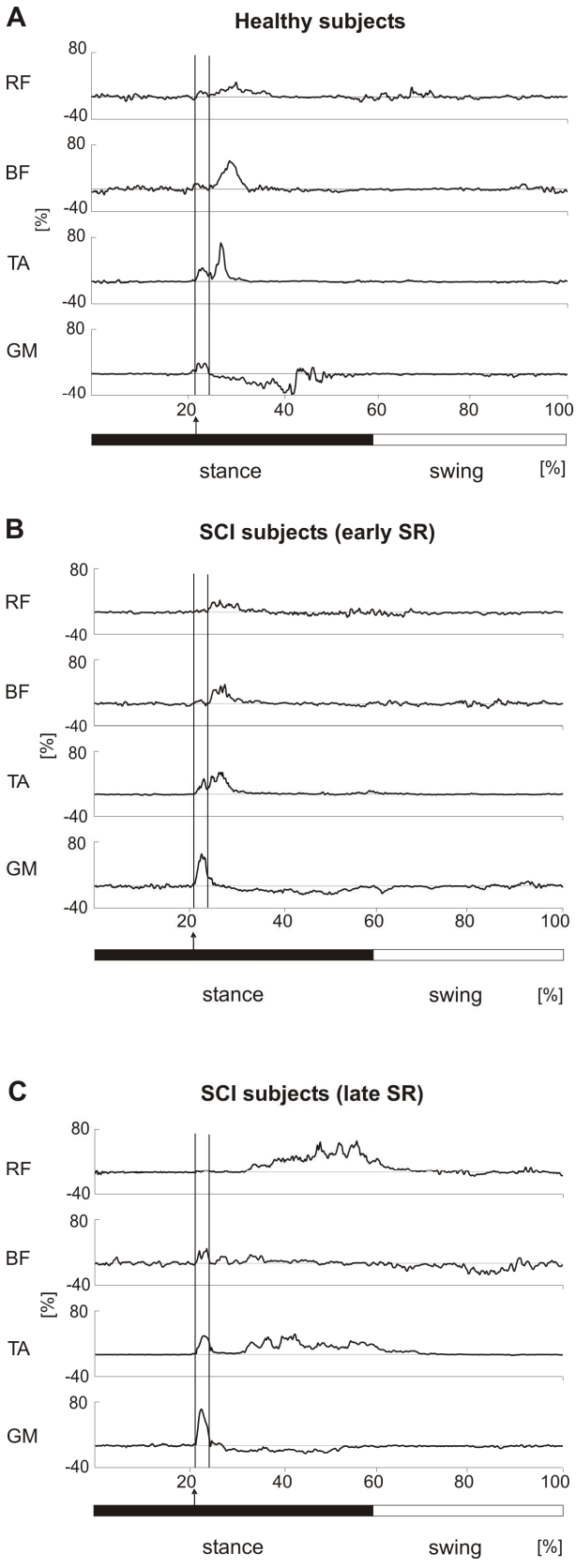

A Healthy subjects
RF
BF
TA
GM
80
0
[%]
500
1000
1500
2000
stance
swing
[ms]
B SCI subjects (early SR)
C SCI subjects (late SR)
stimulated step
non - stimulated step

Fig. 4.2: Influence of SR on the locomotor EMG pattern. The influence of SR released by tibial nerve stimulation on the locomotor EMG activity is shown by median values of healthy (A), cSCI subjects with dominant early SR component (B) and cSCI subjects with dominant late SR component (C). EMG phases are shown by data calculated as averaged (10 gait cycles) leg muscle EMG activity of non-stimulated, normal steps (in gray) and stimulated steps (black). Muscle activity was normalised to the peak amplitude of the stimulated condition. N = 10 for healthy subjects, n = 15 for cSCI subjects with an early SR and n = 5 for cSCI subjects with dominant late SR. Muscle activity was recorded from four leg muscles: rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA) and gastrocnemius medialis (GM). Vertical arrows indicate onset of stimulation, followed by stimulation artifact (two thin parallel vertical lines).


A
Healthy subjects
RF
BF
TA
GM
80
-40
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60
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100
stance
swing
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B
SCI subjects (early SR)
RF
BF
TA
GM
80
-40
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80
100
stance
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C
SCI subjects (late SR)
RF
BF
TA
GM
80
-40
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20
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60
80
100
stance
swing
[%]

Fig. 4.3: Net leg muscle activity of the influence of SR on the locomotor pattern. Recordings of the median net muscle activity of the 3 subject groups are shown: healthy subjects (A), SCI subjects with a dominant early SR component during assisted locomotion (B) and those with a dominant late SR component (C). Net muscle activity of the four ipsilateral leg muscles was calculated as EMG activity of the stimulated condition minus the EMG activity level of the non-stimulated condition (background EMG activity). Vertical arrows indicate onset of stimulation, followed by stimulation artifact (two thin parallel vertical lines).

Figure 4.4 shows the quantified values of net muscle activity and the differences between healthy, cSCI subjects with dominant early or late SR components. Healthy and cSCI subjects with a dominant early SR component showed equal modulation of the locomotor EMG pattern by the early SR component and therefore no difference in their net muscle activity of all muscles could be found. In contrast, the cSCI subjects with dominant late SR components had a significant higher net muscle activity in the RF in contrast to healthy ($p = 0.005$) and cSCI subjects with dominant early SR components ($p = 0.011$). In addition, BF activity was significantly increased in healthy subjects compared to cSCI subjects with dominant late SR components ($p = 0.040$). As described in Figure 4.2 and 4.3, the cSCI subjects with dominant late SR component showed a strong and long-lasting TA activation after SR elicitation. This increased TA net muscle activity was higher than in healthy ($p = 0.055$) and cSCI subjects with early component ($p = 0.053$), however, not significantly different. In the contralateral leg muscles no significant differences between the groups were found.

Background activity did not differ significantly between the two cSCI subject groups (early vs. late SR), neither for the upper (RF $p = 0.284$, BF $p = 0.564$) nor for the lower (TA $p = 0.187$, GM $p = 0.248$) leg muscles. In addition, background activity did not correlate significantly with the SR amplitude in TA of all healthy and cSCI subjects ($n = 30$, $p = 0.110$, $rho = -0.368$).

4.5 Discussion

The aim of this study was to investigate the behaviour of the different SR components, i.e., in which way they influence the locomotor EMG pattern induced by assisted locomotion in cSCI subjects. In earlier studies it was shown that an early SR component dominates in acute and mildly affected SCI subjects and a late one in chronic severely affected SCI subjects (Dietz et al., 2009). The main findings are the following: (i) by the same mode of electrical tibial nerve stimulation two different neuronal networks become activated in cSCI subjects, reflected in the early and late SR; (ii) during assisted locomotion the early and late SR components have a differential effect on the locomotor EMG pattern during the stance phase: the early SR component has a short-lasting effect, while the late SR component causes long-lasting changes of the pattern of leg muscle EMG activity. These findings will be discussed in the context of the physiological roles of the two SR components in relation to the activity of spinal locomotor circuitries.

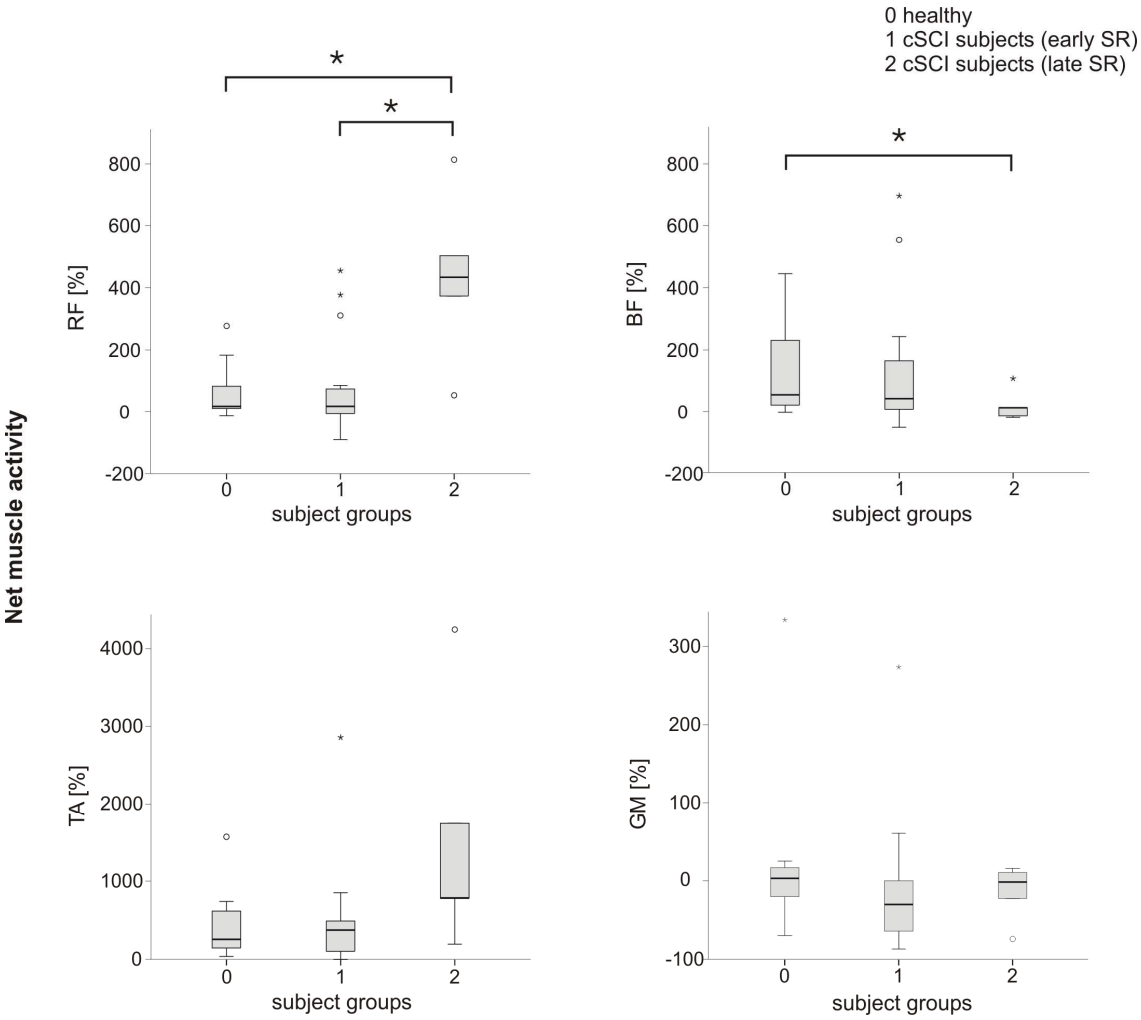

Fig. 4.4: Group differences in net leg muscle activity. Boxplots for four ipsilateral muscles in healthy subjects (subject group 0, n = 10), cSCI subjects with dominant early SR (subject group 1, n = 15) and cSCI subjects with dominant late SR (subject group 2, n = 5) are depicted. Asterisks above the columns define the level of significance of between-group comparisons (*, $p < 0.05$).

4.5.1 Influence of SR on locomotor EMG pattern

Around 2 - 3 months after a cSCI, an appropriate locomotor EMG pattern can be recorded with a normal temporal activation of flexor and extensor muscles but with a strongly reduced EMG amplitude compared to healthy subjects (Dietz et al., 1994, Dietz and Harkema, 2004, Dietz and Muller, 2004). Neuronal circuits underlying the SR are assumed to be part of the locomotor pattern generator in humans (Bussel et al., 1989, Nicol et al., 1995, Parise et al., 1997, Pierrot-Deseilligny and Burke, 2005, Roby-Brami and Bussel, 1987). The observations made with respect to the recovery of both SR and locomotor activity to epidural stimulation in spinal rat (Lavrov et al., 2006) are similar as those described for tibial nerve stimulation and EMG recordings in human SCI (Dietz et al., 2009). Therefore, a close relationship between the activity of SR and that of spinal locomotor circuitries seems to exist in rodent and human SCI. The first polysynaptic SR recorded in spinal rats which correspond to our early SR, was suggested to provide a measure for the restoration of spinal neuronal circuits underlying locomotion (Lavrov et al., 2006). Correspondingly, in chronic incomplete SCI subjects the presence of an early SR component was associated with a preserved walking ability (presence of early SR component also in healthy subjects), while a dominance of a late SR component was characteristic for non-ambulatory SCI subjects and was associated with a neuronal dysfunction during assisted locomotion (Dietz et al., 2009).

Our findings indicate that early and late SR components reflect the activity of separate neuronal circuitries influencing spinal locomotor circuitries in different ways. In subjects with a dominant early SR component during locomotion (regardless of whether it was a healthy or a cSCI subject) the locomotor EMG pattern was only briefly interrupted. The short activation of both flexor muscles (TA and BF) might present a corrective reaction to the tibial nerve stimulation at mid-stance phase. In contrast, cSCI subjects with dominant late SR components a long-lasting interruption of the locomotor EMG activity occurred. In this condition the ipsilateral TA and RF muscles were co-activated from mid- to end-stance. This mode of influence appears not to be physiological as this interruption of locomotor EMG pattern can hardly be interpreted as a corrective reaction.

The different influence of the early and late SR components with the locomotor EMG pattern might be attributed to a difference in motoneuron excitability in the cSCI subject goups (early vs. late). However, this is rather unlikely because there was no difference in

background EMG for all leg muscles between the groups. In addition, the amplitude of the SR components in TA did not correlate with the background TA EMG level.

It is assumed that the early SR component is associated with largely “preserved” function of spinal locomotor circuitries corresponding to spinal rats (Lavrov et al., 2006) and SCI subjects early after injury (Dietz et al., 2009). The dominance of a late SR component in human SCI was assumed to reflect a dysfunction of spinal neuronal circuits below the level of lesion deprived of supraspinal input (Dietz, 2010, Hubli et al., 2011a). In vertebrates it is assumed that the locomotor pattern is shaped by balanced excitatory and inhibitory interneuronal networks integrated into the pattern-generating circuits (Grillner et al., 1995, Guertin, 2009). Our findings are in line with the assumption that the neural circuitries underlying the activity of early and late SR components have a differential effect on shaping the locomotor pattern (Dietz, 2010). In chronic human cSCI an imbalance of locomotor shaping circuitries might develop due to the immobility connected with a loss of an appropriate afferent input (Dietz, 2010).

This would be in line with finding in spinal cats or rats showing that an imbalance between excitatory an inhibitory parts of spinal locomotor circuitries develops (Kitzman, 2006, Tillakaratne et al., 2002). By a locomotor training, however, the balance can be maintained in neonatal transected rats (Ichiyama et al., 2011).

In conclusion, the new findings of this study are that early and late SR components differentially influence the locomotor EMG pattern. Further studies are required to investigate the nature of the spinal neuronal circuitries underlying the SR components leading to the different effects. We assume that chronic animal models of SCI could elucidate the role of the two SR components in spinal locomotion generation.

5 Review 1: Neuronal dysfunction in chronic spinal cord injury [1]

5.1 Abstract

This review describes the changes of spinal neuronal function that occur after a motor complete spinal cord injury (cSCI) in humans. In healthy subjects polysynaptic spinal reflex (SR) evoked by non-noxious tibial nerve stimulation consist of an early SR component and rarely a late SR component. Early after a cSCI, SR and locomotor activity are absent. After spinal shock however, an early SR component reappears associated with the recovery of locomotor activity in response to appropriate peripheral afferent input. Clinical signs of spasticity take place in the following months, largely as a result of non-neuronal changes. After around one year the locomotor and SR activity undergo fundamental changes, i.e., the electromyographic amplitude in the leg muscles during assisted locomotion exhaust rapidly, accompanied by a shift from early to dominant late SR components. The exhaustion of locomotor activity is also observed in non-ambulatory patients with an incomplete SCI. At about one year after injury in most cSCI subjects the neuronal dysfunction is fully established and remains more or less stable in the following years. It is assumed that in chronic SCI the patients' immobility resulting in a reduced input from supraspinal and peripheral sources leads to a predominance of inhibitory drive within spinal neuronal circuitries underlying locomotor pattern and SR generation. Training of spinal interneuronal circuits including the enhancement of an appropriate afferent input might serve as an intervention to prevent neuronal dysfunction after an SCI.

[1] This review is published in the journal Spinal Cord: Neuronal dysfunction in chronic spinal cord injury. Spinal Cord 49 (2011) 582-7. The authors were Michèle Hubli, Marc Bolliger and Volker Dietz. The manuscript was written by Michèle Hubli and revised by the co-authors.

5.2 Introduction

There are several promising neuroregenerative and neuroprotective treatments that are directed to limit neuronal damage and/or induce neuronal regeneration after a spinal cord injury (SCI) (Buchli et al., 2007, Raineteau and Schwab, 2001, Zeman et al., 2001) and they will enter the clinic within the next decade (Deumens et al., 2005, Dietz and Curt, 2006, Raisman, 2003). However, the auspicious results in animal experiments often cannot be replicated in humans. For example, the application of regeneration-facilitating olfactory ensheathing cells (OEC) led to a recovery of function after an SCI in rodents (Barnett and Chang, 2004, Li et al., 1997, Ramon-Cueto et al., 2000, Richter and Roskams, 2008), but had no or only a poor effect on the neurological deficit in humans with SCI (Chhabra et al., 2009, Curt and Dietz, 2005, Dobkin et al., 2006, Lima et al., 2010, Mackay-Sim et al., 2008) in well controlled (Mackay-Sim et al., 2008) but also rather uncontrolled studies (Chhabra et al., 2009, Curt and Dietz, 2005, Dobkin et al., 2006, Lima et al., 2010).

The main problem is the time of the treatment onset. It is known that early treatment onset may help to prevent scar formation (Klapka et al., 2005) and therefore a part of regeneration could occur before scar formation is established. Another important aspect concerns the intrinsic capacity of central neurons to regenerate which leads to the necessity to treat as early as possible after an SCI. Therefore, the treatments in rat models are usually administered soon after the injury (Houle and Tessler, 2003, Karimi-Abdolrezaee et al., 2006, Nomura et al., 2008, Ye and Houle, 1997). In humans, treatment after the SCI is often delayed because at a later stage the clinical condition is more stable. A preservation of the function of spinal neuronal circuits below the level of lesion, however, is an important prerequisite for the success of any kind of regeneration-inducing therapy (Dietz and Curt, 2006).

Studies with chronic cSCI subjects during the past few years provided some evidence that the function of spinal neuronal circuits below the level of the lesion is impaired (Dietz et al., 2009, Dietz and Muller, 2004). The purpose of this review is to depict the behaviour of spinal neuronal circuits deprived from appropriate supraspinal and afferent input and to discuss potential countermeasures to prevent neuronal dysfunction below the level of lesion in the chronic stage of SCI.

5.3 Neuronal dysfunction after SCI

As spinal shock resolves after an acute SCI the reappearance of polysynaptic SR function is associated with the recovery of locomotor EMG activity in rats (Lavrov et al., 2006) and people with cSCI through assisted leg movements (Dietz et al., 1994, Dietz et al., 2009, Dietz et al., 2002). In humans, during the subsequent weeks, the amplitude of locomotor EMG signals and SR activity increase. However, compared with recordings made in healthy individuals, the amplitude of leg muscle EMG activity evoked during assisted locomotion with body-weight support is much reduced (Dietz and Harkema, 2004, Halder et al., 2006).

The neuronal activities that underlie locomotion and SR reach a steady state some months after a human SCI (Hiersemenzel et al., 2000, Karimi-Abdolrezaee et al., 2006). A cSCI at this stage is characterised by the development of spasticity with exaggerated tendon tap reflexes, increased muscle tone, and spasms. Electrophysiological recordings of polysynaptic SR show successively smaller amplitudes of the early SR component and increasing amplitudes of the late SR component, while H-reflexes are unchanged (Hiersemenzel et al., 2000, Jones and Yang, 1994). At a chronic stage of cSCI only late SR components are observed (see Fig. 5.1).

Secondarily occurring non-neuronal changes, i.e., alteration in muscle mechanic, rather than a neuronal hyperactivity, are assumed to be responsible to increased muscle tone at the subacute stage after the SCI (Dietz et al., 1995, Dietz et al., 1981, O'Dwyer et al., 1996). In addition, the difference in the level of muscle activity between active (movement) and passive (clinical) conditions is smaller in spastic limbs than in unaffected limbs (Ibrahim et al., 1993).

Both, spinal and supraspinal lesions lead to both loss of supraspinal drive and defective use of afferent input. These changes obviously affect the behaviour of short and long-latency SR and lead to paresis and maladaptation of the movement pattern. Changes in the mechanical properties of muscle fibers (Lieber and Friden, 2002) result in spastic muscle tone, which partly compensates for the paresis (Dietz and Sinkjaer, 2007). In patients with incomplete SCI, these non-neuronal changes allow to keep posture during walking, as seen in the spastic movement disorder.

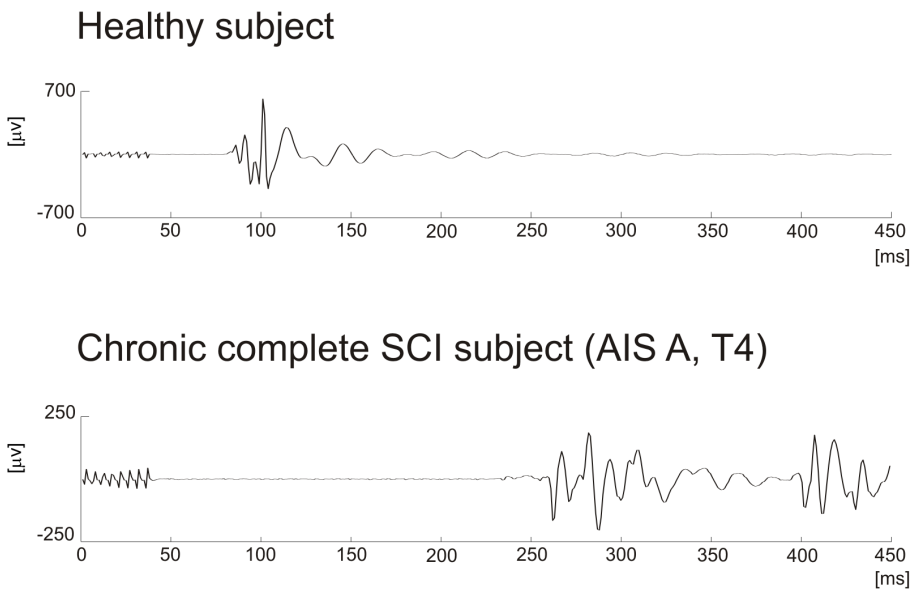

Fig. 5.1: Spinal reflex responses in healthy and chronic cSCI subjects. Representative examples of spinal reflex responses in the ipsilateral tibialis anterior muscle evoked by tibial nerve stimulation at the medial malleolus in a healthy (age 25 years) and a chronic (4.3 years since injury) cSCI (age 23 years) subject. At the onset of the electromyographic recordings, stimulus artifacts are present.

In humans, characteristic changes in neuronal behaviour occur approximately 1 year after a severe SCI: after some minutes of assisted locomotion, the EMG amplitude decreases nearly to a noise level (Dietz and Muller, 2004). This phenomenon called “EMG exhaustion” cannot be reversed by locomotor training and is more pronounced in the leg flexor than in the extensor muscles. So far, no condition in animal models is known that corresponds to the phenomenon of EMG exhaustion in human SCI. The exhaustion of locomotor activity is assumed to take place at a premoto-neuronal (i.e. spinal interneuronal) level (Dietz, 2010). Two observations support this assumption: first, the muscle action potentials and of H-reflexes do not change in amplitude during repetitive nerve stimulation (Dietz and Muller, 2004, Muller and Dietz, 2006) and second, EMG activity of all leg muscles became enhanced during spasms induced by stumbling, despite of an exhaustion of locomotor activity at the end of a training session (Dietz and Muller, 2004).

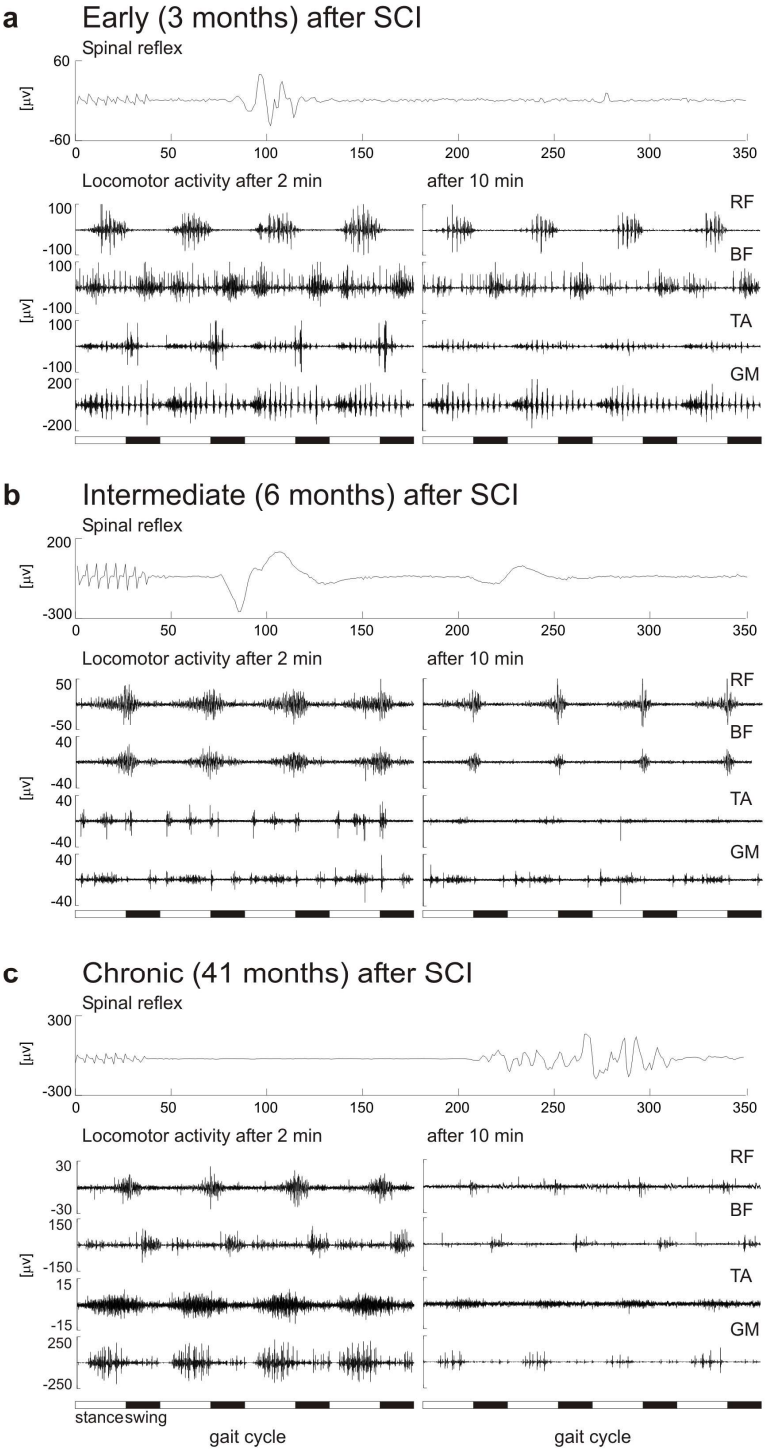

Fig. 5.2: Time course of spinal reflexes and locomotor activity. Representative examples of SR behaviour and locomotor activity during assisted locomotion at (a) early, (b) transition and (c) chronic stages after a complete SCI. Spinal reflexes see Fig. 5.1. Locomotor activity of four leg muscles is shown at the beginning of assisted locomotion (left side) and 10 min thereafter (right side). From (Dietz et al., 2009).

Correspondingly to the EMG exhaustion phenomenon during assisted walking, SR behaviour undergoes changes too in so far that a second late SR component can be evoked by tibial nerve stimulation, while the amplitude of the early component decreases (Dietz et al., 2009). There obviously exists a temporal relationship between the decrease of the early SR component, the increase of the late SR component, and the degree of exhaustion of locomotor activity (Dietz et al., 2009) (see Fig. 5.2).

A partial loss of EMG activity occurs already from the beginning of a training session and encloses a rarefaction of EMG activity / potentials, while during EMG exhaustion the EMG activity is present at the beginning of assisted locomotion and fades out over 5 – 10 min. This partial loss of EMG activity that is observed mainly in the leg flexors seems to occur independently of the exhaustion of EMG activity (Dietz et al., 2009). Trans-synaptic degeneration of motoneurons might be the cause of this loss of EMG activity as suggested by rodent studies (Burns et al., 2005). Neuronal circuits in the spinal cord of rats strongly depend on the input that is lost after an SCI (Ginsberg and Martin, 2002, Wu and Ling, 1998). As well in human SCI there is indirect evidence suggesting a trans-synaptic degeneration of spinal neurons after an SCI (Aisen et al., 1992, Chang, 1998, Lin et al., 2007, Nyboer and Johnson, 1971). For example, using the threshold tracking technique, excitability of peroneal nerve axons was found to drop down distal to the site of injury in SCI subjects (Lin et al., 2007).

The EMG exhaustion phenomenon is observed in the presence of long-lasting immobility, regardless of the completeness of the SCI. While most SCI subjects classified according to the American Spinal Injury Association Impairment Scale (AIS) as AIS A and B show the exhaustion phenomenon (Dietz et al., 2009), patients with sensorimotor incomplete SCI (AIS C and D) who regularly perform stepping movements show no EMG exhaustion, and their early SR component remains dominant. In contrast, incomplete SCI subjects who are wheelchair-bound show the same exhaustion of EMG activity as do AIS A SCI patients and a late SR component is dominant (Dietz et al., 2009).

5.4 Mechanisms of neuronal dysfunction

So far, the cause of the EMG exhaustion phenomenon remains unclear. Our currently favoured assumption is that a loss of supraspinal and appropriate peripheral input causes a predominance of inhibitory influences on the locomotor pattern, leading to the decrease of EMG amplitude during assisted locomotion (Dietz, 2010). In vertebrates,

there exists a close interaction of excitatory and inhibitory interneuronal activity within the pattern-generating neuronal circuits shaping the locomotor pattern (Grillner et al., 1995). After an SCI the excitatory neuronal circuits become deprived of appropriate afferent input and concurrently more centrally controlled inhibitory neuronal circuits might become predominant, resulting in a weakening of the function of excitatory neuronal circuits. These changes might be reflected in the facilitation of long-latency pathways that mediate inhibitory signals, resulting in the inhibition of the early and thereby mediating the late SR component. Indeed, such basic changes in the balance between excitatory and inhibitory inputs to spinal neuronal circuits have been described in cats with SCI (Ichiyama et al., 2006, Tillakaratne et al., 2002) in which walking function could markedly be improved by blocking of inhibitory transmission (de Leon et al., 1999).

The two following observations support the evidence that changes in locomotor and SR function in chronic SCI are caused by a shift towards the predominance of inhibitory neuronal circuits and not necessarily to a degradation of spinal neuronal function: first, the intact locomotor networks even more than 25 years after an SCI, and second, modifiability of EMG exhaustion (and SR changes) in incomplete SCI subjects by functional training.

To what extent an improvement in locomotor function by training is associated with a shift to a dominant early SR component is yet unclear. In addition, it would be of interest to see whether the SR component pattern can serve as a marker to identify preserved locomotor function, i.e., daily walking ability in patients suffering an SCI.

5.5 Potential countermeasures

The preservation of neuronal function below the level of lesion is of crucial importance for the success of future regeneration-inducing treatments after a severe SCI. Presently, regeneration-inducing therapies should not be performed in chronic SCI subjects showing a neuronal dysfunction until appropriate countermeasures are developed. The weak effect of a non-functional recovery described recently for single chronic subjects after OEC transplantation (Lima et al., 2010) would even be compatible with the neuronal dysfunction described here. In the future an appropriate timing of combined regeneration- and plasticity-enhancing therapies should be taken into account (Garcia-Alias et al., 2009, Maier et al., 2009). Functional training seems to have an important role in the prevention of neuronal dysfunction. However, the exhaustion phenomenon could not yet be reversed by a locomotor training in the chronic stage of a motor complete SCI

(Dietz and Muller, 2004). Preliminary observations indicate that the neuronal dysfunction is partially reversible in non-ambulatory AIS C subjects by locomotor training combined with functional electrical stimulation (unpublished observation). In fact, an improvement of ambulatory function through intensive locomotor training can be achieved in chronic incomplete SCI subjects (Wirz et al., 2005). By such a functional training therapy inhibitory signals might become blocked as demonstrated in the cat (de Leon et al., 1999).

Actually several questions have to be answered. How soon after an SCI should be started with locomotor training in order to prevent neuronal dysfunction? Which qualitative features does the training require? Would the provision of some artificial afferent input, for example, repetitive electrical stimulation of flexor-reflex afferents and/or cutaneous afferents, be sufficient to prevent neuronal dysfunction? The aim has to be to induce regeneration before a neuronal dysfunction is established.

5.6 Conclusions

A dysfunction of spinal neuronal circuits underlying locomotion and associated reflex develops after a severe SCI. The dysfunction of spinal neurons chronically deprived of supraspinal and appropriate peripheral input is reflected in an exhaustion of locomotor EMG activity with an associated shift from dominant early to dominant late SR components. These changes are suggested to be due to an imbalance of excitatory and inhibitory neuronal circuits resulting in the emergence of a bias to inhibitory signals within spinal neuronal circuitries. Further research is required to more precisely define the nature of the neuronal dysfunction, i.e., the changes of excitatory and inhibitory activity of neuronal circuits below the level of lesion. In addition, an animal model of chronic SCI might be able to elucidate the neuronal mechanisms underlying the neuronal dysfunction after an SCI and facilitates the development of appropriate countermeasures.

6 Study 4: Spinal neuronal dysfunction after stroke [4]

6.1 Abstract

Background: In chronic spinal cord injured (SCI) subjects a spinal neuronal dysfunction develops this is reflected as an exhaustion of leg muscle electromyographic (EMG) activity during assisted walking associated with a replacement of an early spinal reflex (SR) by a late SR component.

Objective: The aim of this study was to investigate whether a corresponding dysfunction of spinal neuronal circuits, deprived of unilateral supraspinal input after a severe stroke, develops.

Method: In 30 hemiparetic stroke subjects, locomotor ability and SR behaviour in the ipsilateral tibialis anterior muscle, evoked by non-noxious tibial nerve stimulation to both legs, were assessed. In addition, leg muscle EMG activity in nine stroke subjects was recorded during assisted walking.

Results: Similarly to SCI subjects, in severely affected chronic (> 12 months post-incidence) stroke subjects a late SR component was prominent in the affected leg, while an early one dominated in the unaffected leg. The late SR component correlated with muscle paresis (rho = 0.714) and walking ability (rho = 0.493). In contrast to SCI subjects, no exhaustion of the EMG activity was observed in the affected leg muscles during prolonged assisted walking.

Conclusions: It is concluded that spinal neuronal circuits undergo functional changes also after a stroke with common and divergent features compared to SCI subjects. Therefore, neuronal circuits deprived of supraspinal input may function differently, depending on the lesion location, and as a consequence they likely require different rehabilitative strategies clinically.

[4] This manuscript is under review in the journal Experimental Neurology: Spinal neuronal dysfunction after stroke. The authors were Michèle Hubli, Marc Bolliger, Esther Limacher, Andreas Luft and Volker Dietz. Data was assessed and analysed by Michèle Hubli and Esther Limacher. The manuscript was written by Michèle Hubli, Marc Bolliger and Volker Dietz. Revisions were done by the co-authors.

6.2 Introduction

Locomotor activity is generated at the spinal level (Grillner et al., 1981) with both legs closely interacting (Dietz et al., 1989). As this spinal machinery is controlled by supraspinal centres (Iglesias et al., 2008, Schubert et al., 1997), people suffering spinal cord injury (SCI) or stroke experience impaired locomotion.

During the last few years evidence for a spinal neuronal dysfunction below the level of lesion in chronic SCI subjects was found. This dysfunction develops during the first year after a severe SCI and is characterised as both an exhaustion of the EMG during assisted locomotion and a shift from a dominant early (60 - 120 ms latency) to a dominant late (120 - 450 ms latency) spinal reflex (SR) component (Dietz, 2010, Hubli et al., 2011a). SR was defined as a polysynaptic reflex most probably elicited by stimulating cutaneous afferents. Recordings of SR have been used for many years to investigate the function of interneuronal circuits involved in spinal locomotion in mammals (Jankowska, 2001, Kiehn, 2006, McCrea, 2001). For example, a close relationship between the time course of the behaviour of SR and locomotor activity after an SCI was demonstrated in rats (Lavrov et al., 2006) and humans (Dietz et al., 2009). Recently it has been shown that the SR can be used as a marker for the functional state of spinal locomotor circuitries, e.g., only severely affected SCI subjects unable to walk show dominant late SR components (Hubli et al., 2011b). It has been concluded that the development of a spinal neuronal dysfunction is more dependent on the immobility of the subject than on the completeness of the injury. The term 'early and late spinal reflex components' will be applied here as previously in SCI subjects (Bolliger et al., 2010, Dietz et al., 2009, Hubli et al., 2011b). A spinal origin of the latter components can of course not be verified in stroke subjects.

Stroke and SCI subjects share several clinical and functional features, for example, increased muscle tone leading to a spastic movement disorder and exaggerated stretch reflexes. In contrast to SCI subjects, a neuronal interaction between the unaffected and affected leg might prevent the development of a neuronal dysfunction in stroke subjects (Kloter et al., 2011, Reisman et al., 2007) although neuronal links between the two legs and between the arms and legs have been reported to be impaired in stroke subjects (Debaere et al., 2001, Kline et al., 2007).

The aim of this study was to evaluate to what extent spinal neuronal circuits unilaterally deprived of supraspinal input, e.g., after a stroke, develop a dysfunction corresponding with that described after SCI. It is hypothesised that spinal neuronal dysfunction does

not develop due to a protective influence from the unaffected on the affected side and to some preserved non-crossed corticospinal tract fibers (Lemon, 2008). In addition, even in severely disabled stroke subjects some residual locomotor ability remains due to compensatory actions of the unaffected leg, i.e., the affected leg is not completely immobilised.

6.3 Methods

6.3.1 General procedures and subjects

The study protocol was approved by the local ethics committee and conformed to the Declaration of Helsinki. All participants gave informed, written consent before data collection. SR recordings and assessments of mobility and muscle strength were collected from 30 stroke subjects (9 women, 21 men) with a mean age of 56.7 years (range: 25.2 - 80.7 years) and a time since stroke ranging from 1 month up to more than 13 years. For comparison with SCI subjects in which a spinal neuronal dysfunction develops only in the chronic stage, i.e., > 12 months post injury (Dietz, 2010, Hubli et al., 2011a), two cohorts of stroke subjects were investigated: one included 19 chronic subjects (> 12 months post injury) and the other one 11 acute subjects (≤ 12 months). All included stroke subjects showed varying degrees of spastic paresis on the affected side (a few were on small doses of Baclofen medication) but had no major neuro-psychological deficits. The clinical data of all subjects are given in Table 6.1.

Clinical assessments of mobility, muscle strength and SR recordings were performed during a first session (n = 30). Leg muscle EMG activity during assisted locomotion could only be recorded in 9 stroke subjects due to problems in either cardiovascular circulation and/or willingness to participate. This was done during a second (bilateral stepping movements, n = 7) and, in some subjects, a third session (bilateral and unilateral stepping movements, n = 4; two subjects participated in both uni- and bilateral stepping movements) (cf. Table 6.1). Locomotor EMG activity was recorded in order to observe an exhaustion of EMG activity during prolonged assisted locomotion as in SCI subjects (Dietz et al., 2009, Dietz and Muller, 2004).

6.3.2 Assessments of mobility and muscle strength

To assess mobility, subjects performed a 10 meter walking test (10MWT) at preferred speed and were scored with the Functional Ambulation Category (FAC) (Holden et al., 1984). The FAC is scaled from 0 (patient cannot walk or needs the help of two or more

Subject	Age (years)	Gender	Affected side	Duration of stroke (months)	Locomotor activity
S1	51.3	m	left	12	+[1]
S2	75.8	f	right	36	+[1]
S3	63.9	m	left	43	+[2]
S4	80.7	m	left	21	+[1]
S5	79.7	m	left	40	
S6	69.5	f	right	100	+[1,2]
S7	60.5	m	right	26	+[1]
S8	40.2	m	left	80	+[1]
S9	45.8	m	right	12	+[1,2]
S10	59.8	f	left	26	
S11	60.3	f	left	23	
S12	44.6	f	right	28	
S13	54.3	f	right	87	
S14	52.3	f	right	5	+[2]
S15	47.0	m	left	24	
S16	43.8	m	left	5	
S17	65.0	m	right	119	
S18	54.8	m	left	5	
S19	46.1	m	right	166	
S20	59.1	f	left	37	
S21	56.8	m	right	2	
S22	61.8	m	right	8	
S23	49.3	m	right	5	
S24	25.2	m	right	37	
S25	70.8	m	left	180	
S26	62.1	w	right	28	
S27	64.7	m	right	7	
S28	44.3	m	left	35	
S29	43.8	m	left	1	
S30	66.5	m	left	1	

Tab 6.1: Characteristics of the stroke subjects included in the study. The recordings made during 1: bilateral and 2: bilateral and unilateral step-like leg movements with body weight support (60% BWS) were performed only in selected subjects.

persons) to 5 (patient can walk anywhere without help), depending on the amount of personal help required to keep balance and coordination. In addition, subjects were asked to complete the Rivermead Mobility Index (RMI), a questionnaire concerning the subjects' own ability to move (Collen et al., 1991). This test includes 15 questions where an answer of "yes" is scored as one point. The total RMI score ranges from 0 for no mobility to 15 for good mobility.

Muscle strength of both legs was tested using the Motricity Index (MI) for lower limbs (Collin and Wade, 1990). Ankle dorsiflexion, knee extension and hip flexion movement were evaluated separately. For the three movement tasks there was a maximum score of 100 for each leg.

6.3.3 Spinal reflex recordings

SR were elicited in a supine position by electrical stimulation of the distal tibial nerve using a constant current stimulator (AS 100, ALEA Solution GmbH, Zurich, Switzerland). Bipolar surface stimulation electrodes (Ambu Neuroline 700, 15x20mm, Ambu A/S, Ballerup, Denmark) were placed posterior to the medial malleolus of both feet. The stimulus consisted of a train of eight biphasic rectangular pulses with a single stimulus duration of 2 ms and a frequency of 200 Hz (Muller and Dietz, 2006) resulting in a total stimulation duration of 40 ms. SR threshold was determined by a gradual increase in stimulation intensity by 2 mA steps up to the first visible contraction of the abductor hallucis muscle (motor threshold) (Hiersemenzel et al., 2000). Stimulation intensity was set to double the motor threshold. In healthy subjects, this stimulation intensity has been reported to be non-noxious (Dietz et al., 2009). Mean stimulation intensity was 16.0 mA (sd = 6.5 mA) for the affected side and 15.8 mA (sd = 8.1 mA) for the unaffected side. Both, determination of threshold and release of a SR were done in a supine position.

The SR evoked by this stimulation protocol is most probably mediated by group II afferents. Although we use the term 'spinal reflex', we cannot exclude, that later parts of the SR (> 120 ms latency) are mediated by supraspinal pathways, since in stroke subjects (in contrast to complete SCI subjects where the stimulation protocol was applied earlier) some supraspinal input is preserved.

Five electrical stimulations were randomly applied to each leg, i.e., electrical stimuli were released with an interval of 30 - 45 sec to avoid habituation (Fuhrer, 1976), and the EMG responses of the ipsilateral tibialis anterior (TA) muscle were recorded using dual

surface electrodes (Noraxon, Cologne, Germany). The EMG signals were amplified, filtered (bandpass 30 - 300 Hz) and sampled at 1000 Hz via a 12-bit A/D-converter.
SR responses in the TA muscle of both sides were analysed for the presence of early and late components. Time windows were set from 60 - 120 ms after stimulation onset for the early and from 120 - 450 ms for the late SR component (Muller and Dietz, 2006, Pierrot-Deseilligny and Burke, 2005). SR analyses were done as described earlier (Dietz et al., 2009). SR analysis resulted in a score between 1 and -1 for normalised early minus late SR component. Positive values indicate the dominance of early SR components, negative values indicate the dominance of the late SR components, and the value 0 means that early and late SR components are equally dominant.
Scores of functional tests and SR analyses are given in Table 6.2.

6.3.4 Locomotor EMG pattern

Assisted locomotion was achieved by the driven gait orthosis (DGO) 'Lokomat' (Hocoma AG, Volketswil, Switzerland) mounted on a treadmill (Colombo et al., 2000). The DGO moved the subject's legs along a pre-defined trajectory. Passive foot-lifters (elastic straps) prevented stumbling during the swing phase. Subjects were connected by a harness to a body weight support (BWS) system and walked with 55 - 65 % BWS to enable a physiological gait pattern.
Besides normal bipedal locomotion the EMG activity during unilateral walking was assessed. Unilateral walking was recorded to examine whether an EMG exhaustion develops in the affected leg muscles without a potential influence of the unaffected leg. Unilateral stepping movements were induced by static positioning of the unaffected leg in front of the subject by a strap around the thigh. For each condition (bilateral and/or unilateral stepping leg movements), subjects walked for 10 - 15 min in the DGO. The amount of BWS and treadmill speed at 2.0 km/h were kept constant.
EMG signals of proximal and distal muscles of both legs, i.e., rectus femoris (RF), biceps femoris (BF), TA and gastrocnemius medialis (GM), were recorded using dual surface electrodes (Noraxon, Cologne, Germany). In addition, the left and right heel strikes were recorded and were used as trigger signals. The data were sampled continuously at 1500 Hz and stored on a hard disk for analysis. For data acquisition and processing, the commercially available software Soleasy (ALEA Solution GmbH, Zurich, Switzerland) was used. The mean EMG amplitude of all leg muscles was determined by calculating the root mean square (RMS) of 10 strides after 2 and 15 min of walking.

Subject	FAC	RMI	MI affected	MI unaffected	10MWT [m/s]	SR affected	SR unaffected
S1	3	10	40	92	54.5	0.87	0.92
S2	3	7	26	84	42.0	-0.79	0.52
S3	4	13	40	100	21.9	-0.28	-0.54
S4	4	11	70	100	13.2	-0.01	0.24
S5	0	6	40	100		0.42	0.60
S6	4	11	29	100	27.8	-0.13	0.64
S7	4	13	39	100	15.1	-0.29	0.41
S8	0	3	15	84		-0.38	0.31
S9	5	14	51	92	12.9	0.12	0.89
S10	2	6	45	92	94.8	-0.66	0.80
S11	5	11	39	92	15.6	-0.50	-0.07
S12	5	13	59	100	13.7	0.19	0.17
S13	4	14	39	100	19.7	-0.42	-0.48
S14	5	12	67	100	10.7	0.65	-0.31
S15	5	13	44	100	23.8	0.17	0.89
S16	4	11	65	100	36.4	0.82	0.83
S17	5	13	54	100	18.0	-0.93	-0.48
S18	5	13	39	100	24.4	-0.51	0.18
S19	0	8	19	100		-0.96	-0.80
S20	1	7	54	100		0.21	0.31
S21	3	6	53	100	25.8	0.14	-0.29
S22	5	14	76	100	11.1	0.68	0.77
S23	5	14	100	100	11.2	0.82	0.89
S24	5	15	100	100	6.53	0.80	0.96
S25	4	13	76	100	9.12	0.87	0.83
S26	5	15	76	100	7.75	0.69	0.91
S27	5	15	100	100	9.22	0.79	0.83
S28	5	13	70	100	8.09	0.26	0.89
S29	2	4	84	100		0.05	0.83
S30	0	2	34	92		0.81	0.60

Tab. 6.2: Data of the assessments collected in the study. FAC = Functional Ambulation Category (healthy: 5); RMI = Rivermead Mobility Index (healthy: 15); MI = Motricity Index (healthy: 100); 10MWT = 10 Meter Walking Test; SR = Spinal Reflex (healthy: 1).

6.3.5 Statistics

Statistical calculations were performed using PASW Statistics 17.0 for Windows (SPSS Inc., Illinois, USA). For the assessment of differences in the amplitude of the EMG values between the affected and unaffected leg and over time during unilateral and bilateral stepping movements, the Wilcoxon Signed Ranks Test was used. The difference of SR in acute and chronic stroke subjects was calculated by the Mann-Whitney U Test. The difference between SR of the affected side and unaffected side was tested using the Wilcoxon Signed Ranks Test. Correlations between the SR in the TA muscle and the assessments for mobility and muscle strength were tested using the Spearman's rank correlation coefficient. Level of significance was set at $p < 0.05$ for all tests.

6.4 Results

6.4.1 Spinal reflex activity

Typical examples of SR responses in the unaffected and the affected leg of three stroke subjects are shown in Figure 6.1. In the TA muscle of the unaffected leg of all three subjects only an early SR component was present. At 12 months after a severe stroke both legs showed early SR responses (Fig. 6.1A). At 24 months after the incident an early and a late reflex component appeared in the affected leg in a mildly affected stroke subject (Fig. 6.1B). However, in a severely affected subject at the corresponding time post-injury (26 months) a long-lasting late SR component appeared in the TA of the affected leg (Fig. 6.1C).

Figure 2 shows the SR behaviour of all stroke subjects included in this study (Fig. 6.2A) and of the affected and unaffected side of all chronic stroke subjects (Fig. 6.2B). There were differences ($p = 0.018$) in SR behaviour of the affected leg between acute (median 0.68, CI ± 0.27) and chronic (median: -0.13, CI ± 0.25) stroke subjects (Fig. 6.2A). In the acute stage after stroke (≤ 12 months) an early SR component dominated, while in the chronic stage a late SR component became prominent. In addition, there was a significant difference between the two legs in the chronic stroke subjects with a predominance of the early SR component in the unaffected side ($p = 0.001$). In contrast, a late SR component was prominent in the TA of the affected leg.

As most acute stroke subjects show no late SR components (comparable to SCI subjects), the relation between SR and mobility assessments were only done for chronic

stroke subjects. The relation between clinical (Fig. 6.3A) and functional (Fig. 6.3B) tests and SR in all chronic stroke subjects is shown in Figure 6.3. The clinical assessment of the leg muscle strength (MI) of the affected side correlated significantly to the relationship between the amplitudes of the early and late SR components (rho = 0.714; p = 0.001). The lower the motor score of the affected leg, the more dominant the late SR component and vice versa. Thus, in most severely affected stroke subjects the late component dominated. The motor score was not related to the chronicity of the stroke.

The walking speed of most subjects was in a narrow range (0.33 - 0.67 m/s) and not all stroke subjects were able to ambulate (0.0 m/s) (Fig. 6.3B). Nevertheless, walking speed was correlated with SR behaviour (rho = 0.493; p = 0.032), i.e., the faster a stroke subject was able to walk the more dominant was the early SR component. The other functional tests (FAC and RMI) showed no significant correlation with SR behaviour.

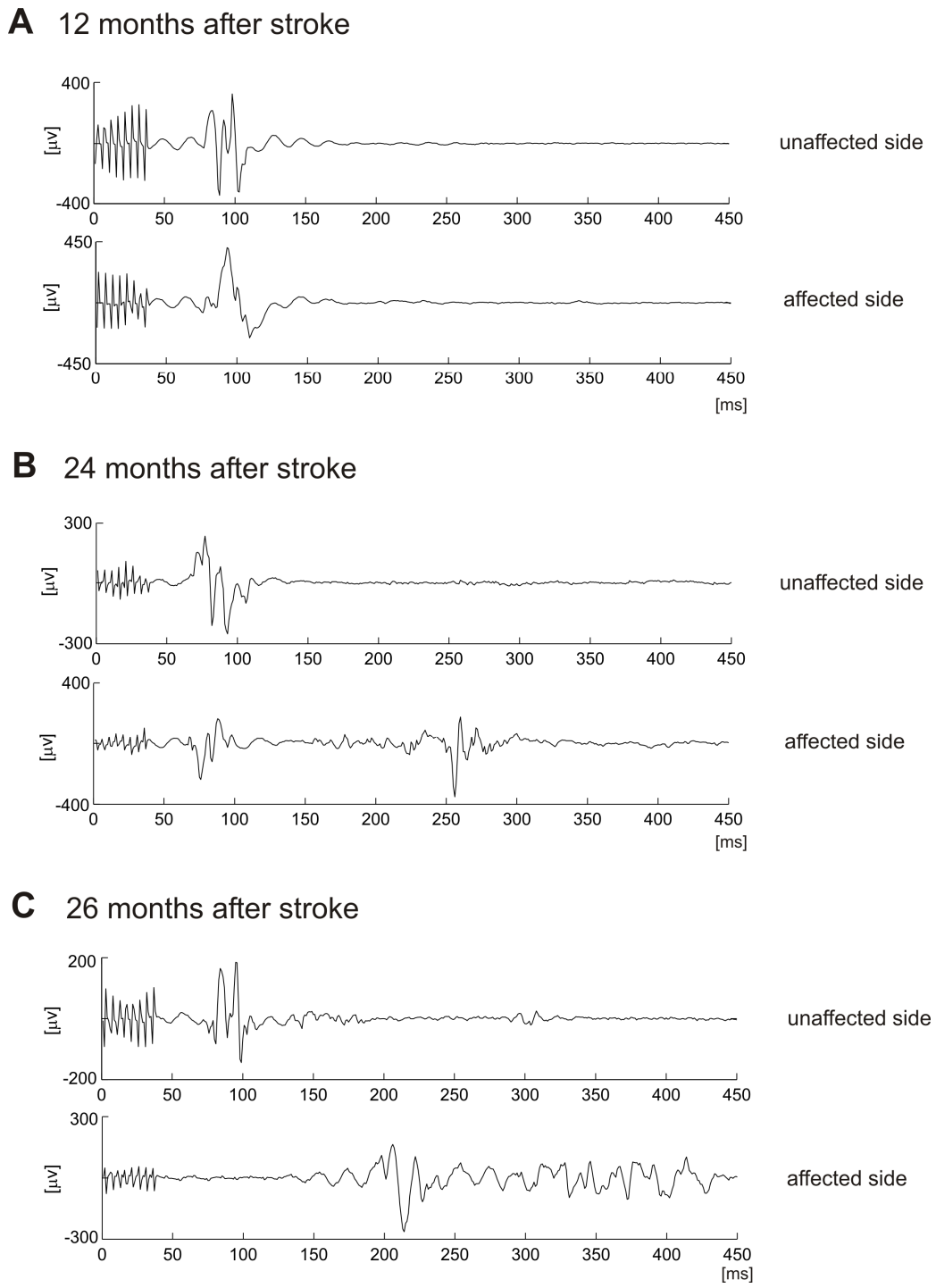

Fig. 6.1: Spinal reflex (SR) components. SR components in the TA muscle of the unaffected and the affected leg of 3 stroke subjects at different stages post-stroke. (A) 12 months (Functional Ambulation Category (FAC 3), (B) 24 months (FAC 5) and (C) 26 months (FAC 2) after stroke.

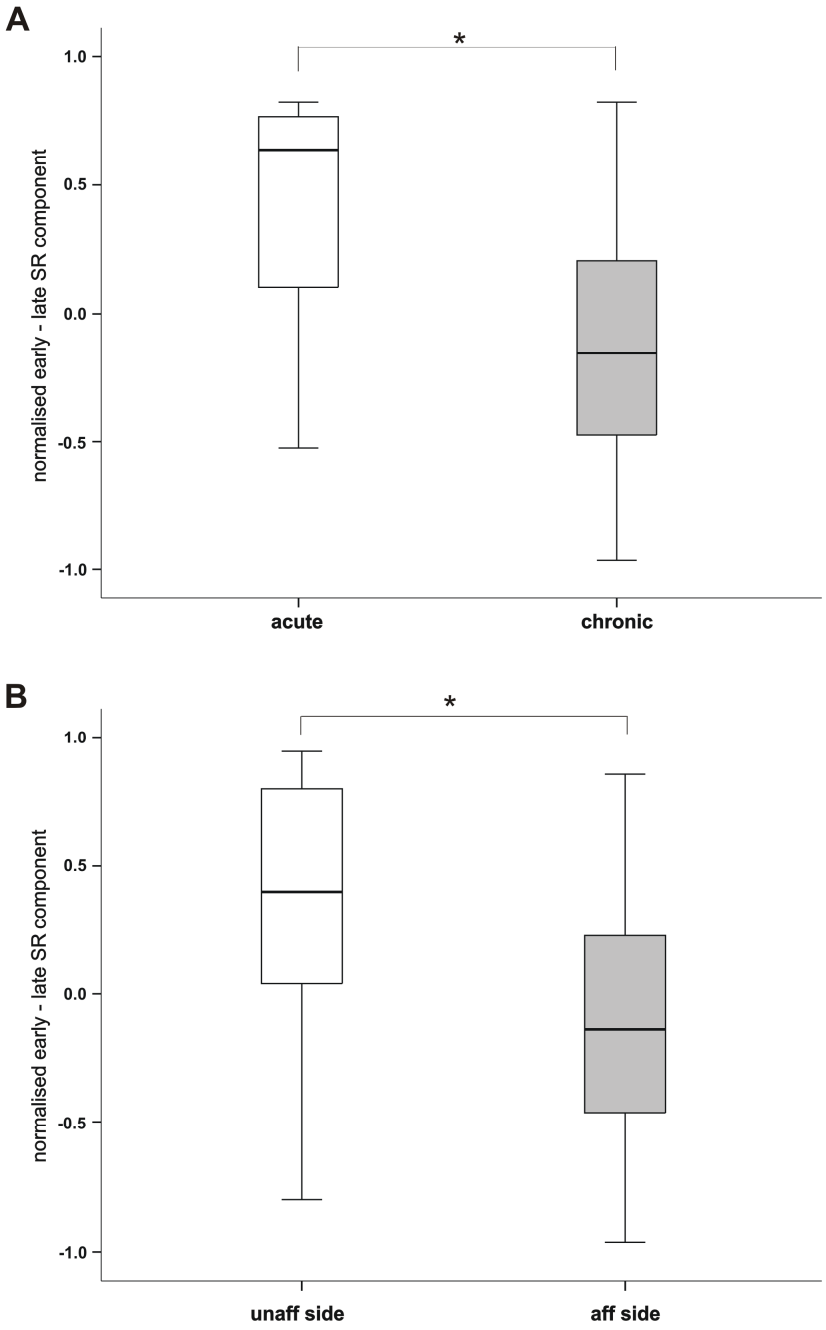

Fig. 6.2: Changes in SR behaviour after stroke. (A) Normalised early minus late SR component of the affected leg at an acute (≤ 12 months after stroke, n = 11) and a chronic (> 12 months after stroke, n = 19) stage. Significant difference is indicated by asterisk (p = 0.018). (B) Normalised early minus late SR component of the unaffected and affected leg from all chronic (> 12 months after stroke) subjects (n = 19). Significant differences are indicated by asterisks (p = 0.001).

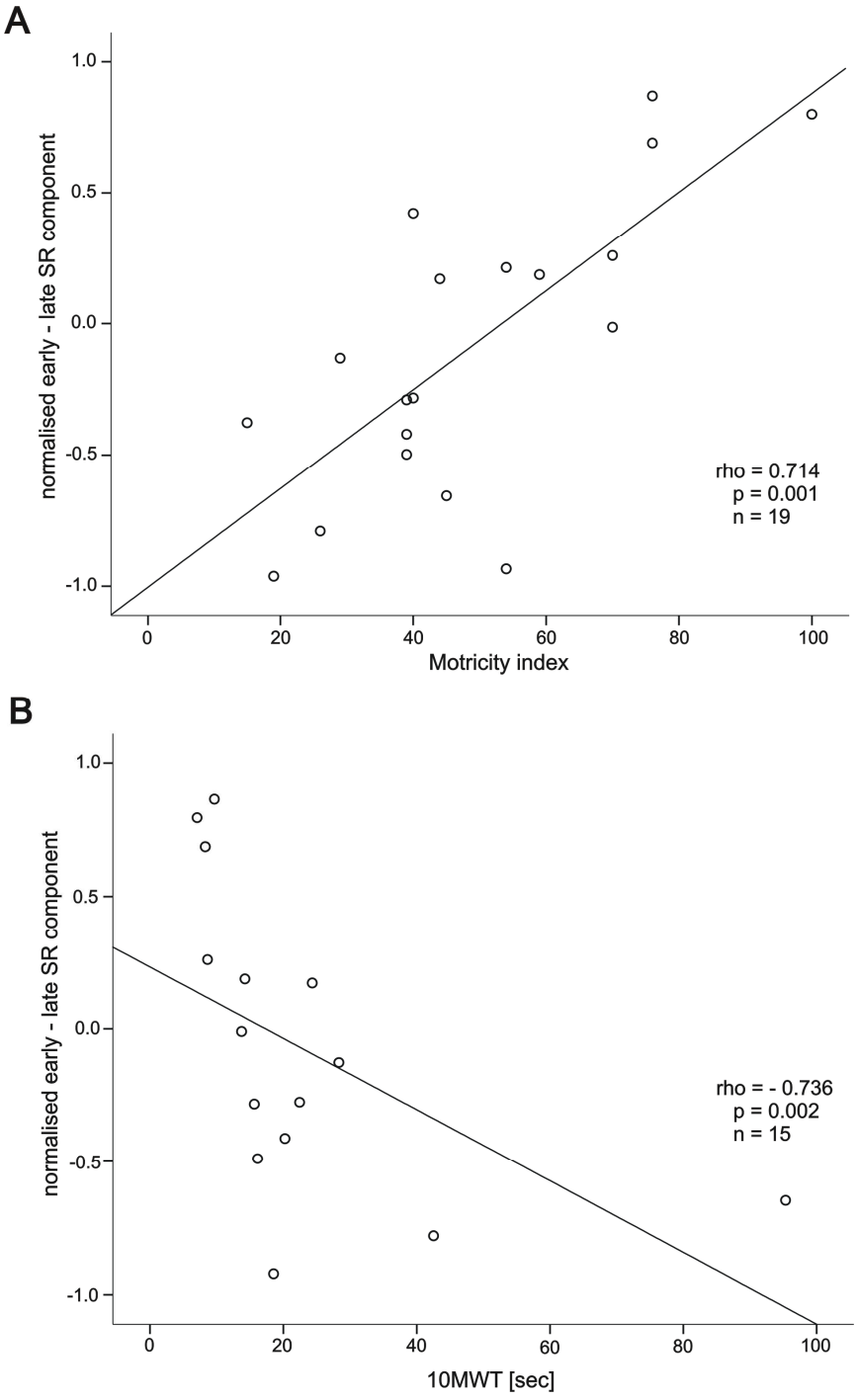

Fig. 6.3: Relation between SR and clinical/functional scores. Relation between normalised values of SR components and (A) clinical assessment of maximal voluntary muscle force of the affected leg (Motricity Index: 0-100) and, (B) walking ability (10 meter walking test) in chronic stroke subjects.

6.4.2 Locomotor EMG activity

Figure 6.4 shows the leg muscle activation pattern of a chronic stroke subject after 2 and 15 min of assisted (bilateral) walking. On the unaffected side (Fig. 6.4A) the leg muscle EMG pattern appears to be normal, i.e., it did not visibly differ from that of healthy subjects (Den Otter et al., 2006). In contrast, on the affected side (Fig. 6.4B) the EMG amplitude was reduced compared to the unaffected leg, but the temporal patterning of EMG activity was preserved. The EMG amplitude was reduced in the affected leg compared to the unaffected leg ($p < 0.05$ for TA and GM).

Changes in leg muscle EMG amplitudes did not occur in either the affected or the unaffected leg muscles during 15 min of assisted locomotion (unilateral and bilateral). Hence, a leg muscle EMG exhaustion was not detected during prolonged assisted locomotion in any of the stroke subjects studied (chronic, severely affected SCI subjects show such an EMG exhaustion after 8 - 10 min of assisted locomotion (Dietz et al., 2009)).

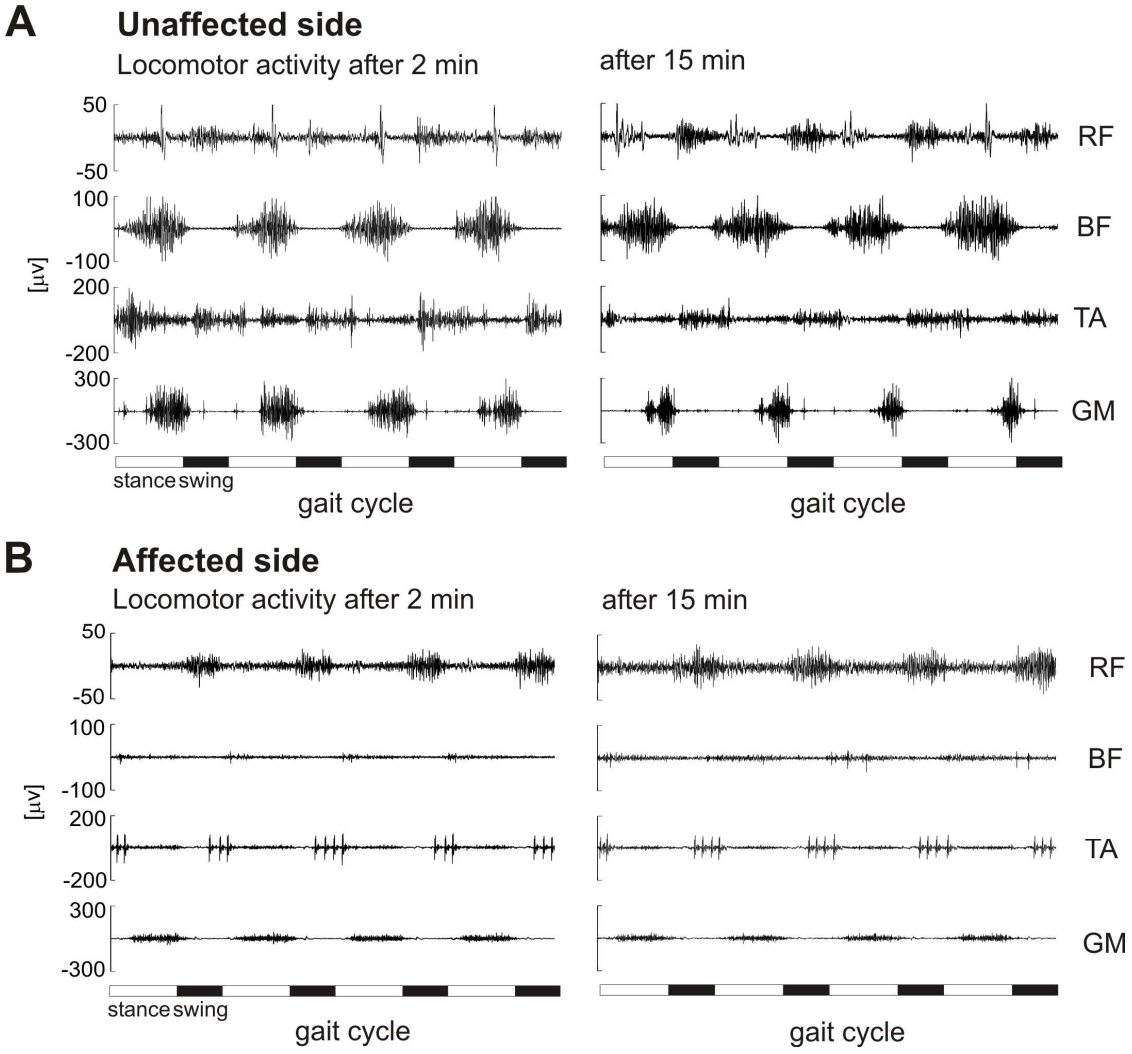

Fig. 6.4: Locomotor activity pattern. Typical pattern of leg muscle activity (RF rectus femoris, BF biceps femoris, TA tibialis anterior, GM gastrocnemius medialis) of (A) the unaffected and, (B) the affected leg of a stroke subject (21 months after stroke) after 2 and 15 min of walking on the treadmill with 2 km/h.

6.5 Discussion

The aim of this study was to evaluate the function of spinal neuronal circuits in subjects deprived of supraspinal input. In chronic severely affected SCI subjects the development of a spinal neuronal dysfunction was described (Dietz, 2010, Hubli et al., 2011a). Here we asked the question whether a similar dysfunction also develops in severely affected stroke subjects. Similarly to SCI, a shift from an early to a dominant late SR component developed in the affected leg of severely disabled stroke subjects, and this was related to both the clinical and the functional deficit. However, in contrast to the situation after SCI, this shift was not associated with a drop in EMG activity, i.e., exhaustion in the affected leg muscles during repetitive steps.

6.5.1 Spinal reflex dysfunction

The neuronal circuits of the reflexes evaluated in this study, which were evoked by non-noxious stimuli whilst in a supine position, are assumed to be closely connected with spinal locomotor circuitries (Dietz et al., 2009, Lavrov et al., 2006). Such a close relationship between SR and locomotor activity has been previously shown for rats (Schouenborg, 2002) and cats (Jankowska and Riddell, 1995). Although the mode of stimulation is different in these animal models compared to the one applied in this study, the long-latency reflex discharges in the cat (Jankowska et al., 1967) and the polysynaptic SR evoked in the rat with a transected spinal cord (Lavrov et al., 2006) are associated with locomotor activity and might correspond to the early SR component described here.

Correspondingly to the findings in SCI subjects (Dietz et al., 2009), a dominant late SR component was present only in the affected leg of all severely disabled chronic stroke subjects. Its dominance was related to the clinical motor deficit and the 10MWT but not to the FAC and RMI. The heterogeneity of the brain lesions (not evaluated) had no major effect on this relationship.

The difference in functional tests might be due to the fact that, in contrast to SCI subjects, walking disorders after stroke are partly compensated by the unaffected leg and, consequently, the impaired function of the affected leg is not sufficiently reflected in subjects' rating of mobility.

The appearance of early and late SR components in the unaffected and affected leg of stroke subjects corresponded to those described in SCI subjects (Dietz et al., 2009).

Therefore, the term ‘spinal reflex’ was also applied here. However, it is of course not possible to determine that the latter reflexes recorded in this study are of spinal origin.

6.5.2 Dysfunction of locomotor actvity

In most of our subjects the leg muscle activation pattern differed considerably between the unaffected and the affected leg. Visibly, the leg muscle activity in the unaffected leg did not differ from that of healthy subjects (Den Otter et al., 2006, Dietz and Muller, 2004). The small EMG amplitude in the affected leg muscles of stroke subjects during locomotion has been described previously (Den Otter et al., 2006).

The difference in the EMG pattern between the leg muscles of the two sides in chronic stroke subjects might be due to the fact that no physiological stepping movements are exerted by the affected leg, because it mainly serves as support of the body, like a cane, during the swing phase of the unaffected leg (Dietz and Sinkjaer, 2007).

6.5.3 Divergent behaviour of SR and locomotor activity

In contrast to SCI subjects, in stroke subjects the impaired leg function and dominant late SR component were not associated with an exhaustion of leg muscle activity during assisted locomotion, even when stepping movements were performed solely by the affected leg. A possible explanation for the lack of EMG exhaustion in severely affected stroke subjects might be due to subliminal influences of the unaffected leg muscle activation on the affected leg. This, together with some training effects on the affected leg muscles, might prevent EMG exhaustion. In similarly affected, sensori-motor incomplete (AIS C) SCI subjects a dominant late SR component and exhaustion in the EMG were only present when subjects were immobilised (Dietz et al., 2009). This was not the case in any of our stroke subjects.

It remains an open question why neuronal dysfunction after stroke has, in contrast to SCI, a different effect on leg muscle EMG exhaustion during assisted walking. Our favored hypothesis is that the EMG exhaustion and the dominance of late SR components reflect two different aspects of a spinal neuronal dysfunction. The mechanisms underlying the dysfunction have to be explored further. For SCI subjects it was assumed that an imbalance between excitatory and inhibitory neuronal circuits towards an inhibitory drive on spinal locomotor circuitries leads to the spinal neuronal dysfunction (Dietz, 2010, Hubli et al., 2011a).

Despite the presence of common clinical characteristics of lesions to the central nervous system, this study provides evidence for lesion-dependent effects of how the deprivation of supraspinal drive leads to dysfunctional spinal neuronal circuits over time post-injury. The therapeutic conclusion from this study would be to apply a focused training on the affected leg, in a similar way to constraint-induced movement therapy of the affected arm in stroke subjects (Taub et al., 1999). By such an approach the affected leg would be trained independently because compensation by the unaffected leg would be prevented. As a consequence of this type of training, the development of a neuronal dysfunction might be avoidable.

7 General Discussion

The aim of this thesis was to investigate changes in spinal neuronal circuits over the course of an SCI in humans with the aim to develop countermeasures to prevent a neuronal dysfunction below the level of lesion in a chronic stage of an SCI. These investigations were addressed in four different studies. In contrast to animal research in this field, we were not able to address changes in spinal neuronal circuits by pharmacological interventions or histological preparations. However, we assessed plasticity of the neuronal networks by indirect measurements of SR function and locomotor EMG activity. Analysis of locomotor EMG pattern provides important information regarding the role of spinal neuronal circuits, and their interaction with afferent input, in the generation of locomotor activity. The analysis of polysynaptic SR provided complementary insights, e.g., information about the organisation of spinal interneuronal circuits.

The *first study* investigated the time course of changes in SR behaviour and locomotor activity after a complete SCI. The *second study* extended these investigations of SR behaviour and locomotor activity to incomplete SCI subjects and analysed the plasticity of SR and spinal locomotor circuitries after locomotor training. The *third study* analysed how the two different SR components (early and late) interact with the locomotor EMG pattern induced by assisted locomotion in the Lokomat in complete SCI subjects. The *fourth study* compared the neuronal dysfunction described in SCI with that occurring after a stroke. The findings of each individual study have been discussed in the specific discussion sections. In the following, the findings are discussed in the conjunction with each other and briefly summarised in the context of respective research questions stated in the general introduction of the thesis. Subsequently, the potential clinical significance and limitations are discussed. Finally, the thesis is closed with the conclusion and an outlook about further investigations.

7.1 Spinal interneurons - changes after SCI

This thesis with its four studies explored changes in spinal interneuronal circuits after central lesions (SCI and stroke) with the goal to find ways to prevent a neuronal degradation below the level of lesion. These investigations are of major interest because a preserved function of spinal neuronal circuitries is a prerequisite for any kind of regeneration-inducing therapies available for SCI subjects in the near future (Curt and

Dietz, 2005). In the first three studies spinal neuronal circuits have been investigated by studying SR behaviour and locomotor activity in different groups of SCI subjects, i.e., motor complete (AIS A/B), motor incomplete (AIS C/D), acute or chronic SCI subjects. A degradation of spinal neuronal function following a complete SCI was described earlier (Dietz and Muller, 2004). This thesis strengthens the evidence of a neuronal dysfunction below the level of lesion. Next to a loss of walking ability after SCI, mainly two aspects have been revealed: first, a shift from dominant early to dominant late SR components in chronic complete SCI and severely affected incomplete SCI and second, a locomotor EMG exhaustion during assisted locomotion. In addition, SR activity reflects the functional state of spinal neuronal circuitries after an SCI, since the SR behaviour is strongly related to the walking ability of chronic SCI subjects. The neuronal dysfunction described starts at around 1 - 2 years after a severe SCI in humans. However, it might not be a fixed state of dysfunction of spinal neuronal circuits. Both, the SR behaviour and the locomotor ability can be positively modified by a locomotor training. Hence, changes in spinal neuronal circuits after an SCI seem to indicate a plastic behaviour at least in incomplete SCI subjects.

Several aspect of this thesis concerning spinal neuronal behaviour should be considered when developing new countermeasures. First, neuroplasticity can have both positive and negative manifestations. This thesis can fortunately report only positive aspects which are desired during rehabilitation processes and reflect the improvement of SCI outcome achieved through functional training in animals (Courtine et al., 2009, Edgerton et al., 1997, Edgerton et al., 2004, Garcia-Alias et al., 2009, Hains et al., 2003) and humans (Behrman and Harkema, 2000, Dietz and Harkema, 2004, Gardner et al., 1998, Hesse et al., 2004, Wernig et al., 1999, Wirz et al., 2005).

Second, the EMG exhaustion phenomenon seems to rather depend on the presence of long-lasting immobility than on the completeness of an SCI. While all motor complete SCI subjects (AIS A/B) show the exhaustion phenomenon, subjects with motor incomplete SCI (AIS C/D) who regularly perform stepping movements show no EMG exhaustion, and the early SR component is dominant (study 1 and 2).

Third, if a neuronal dysfunction is once established in chronic complete SCI subjects, it seems not to be possible to change it (Dietz and Muller, 2004). Although a well-modulated EMG pattern can be induced during the first minutes of assisted locomotion, the spinal locomotor circuitries get more fragile.

7.2 Mechanisms underlying neuronal dysfunction

The cause of the neuronal dysfunction remains unclear so far. As proposed by the first study, we assume common underlying mechanisms for both, the changes in SR behaviour and locomotor activity. However, since in severely affected stroke subjects no EMG exhaustion could be assessed although the late SR component was dominant, one should consider different underlying physiological basis of the two aspects of neuronal dysfunction.

There are two main assumptions explaining the changes in SR behaviour and locomotor activity after an SCI. One potential explanation is that a degradation of spinal neuronal function by anatomical changes takes place in spinal networks after an SCI. Such a mechanism was suggested by the early observation that locomotor training could not reverse the neuronal dysfunction in chronic complete SCI subjects (Dietz and Muller, 2004). However, this thesis report reversing effects on the neuronal dysfunction in severely affected incomplete SCI subjects after locomotor training. This observation led to another, more probable, second hypothesis: The changes in SR and locomotor activity might be ascribed to a synaptic 'fatigue' that results by a shift from balanced excitatory and inhibitory spinal interneuronal activity towards a predominance of inhibitory neuronal circuitries following the loss of appropriate activation of spinal neuronal circuitries in chronic complete and severely affected incomplete SCI subjects.

In the following, the second hypothesis will be further elucidated on the basis of knowledge about excitatory and inhibitory spinal networks in animal models, mainly rats and cats. In lower vertebrates, the locomotor pattern is shaped by excitatory and inhibitory interneuronal activity within the spinal locomotor circuitries (Grillner et al., 1995). Drastic changes in the balance between excitatory and inhibitory activity in spinal locomotor circuitries towards the latter have been described in cats (Tillakaratne et al., 2002) and rats (Ichiyama et al., 2006) with transected a spinal cord. These findings are in line with our assumption that excitatory neuronal function deprived from afferent and supraspinal input weakens, while inhibitory activity become dominant. These changes could represent a basis of the distinct neuronal dysfunction of spinal neuronal circuitries described in this thesis, i.e., inhibition of the early SR component leading to a dominance of late SR components and decrease of locomotor activity. Through an appropriate locomotor training in incomplete SCI subjects the balance between excitatory an inhibitory input to motoneurons might be regained, as it has been shown to occur in neonatal transected rats (Ichiyama et al., 2011).

The possible function of the early and late SR components can be suggested on the basis of the investigation of the interaction of the two different SR components (early and late) with the locomotor EMG pattern during assisted locomotion in complete SCI subjects (study 3). In this study it is concluded that the same tibial nerve stimuli activated two different neuronal pathways, resulting in divergent interactions with spinal locomotor circuitries. Therefore, it is assumed that the two SR components have different physiological roles during locomotion: the early SR component might be associated with largely preserved or regained function of spinal locomotor circuitries corresponding to spinal rats (Lavrov et al., 2006) and SCI subjects early after injury (study 1), or after locomotor training (study 2). The dominance of the late SR component in chronic complete SCI subjects is assumed to reflect a dysfunction of spinal neuronal circuits below the level of lesion deprived of supraspinal input (Dietz, 2010, Hubli et al., 2011a).

The comparison of SR components in animal models and humans has been made, although different stimulation protocols have been used (see 7.4 Limitations). One important publication concerning the relationship between SR and locomotor activity in rat is that of Lavrov et al. (2006). Corresponding to human SCI (Hiersemenzel et al., 2000), the polysynaptic SR is lost during the first phase after spinal cord transection in rats, but is restored several weeks later (Lavrov et al., 2006, Valero-Cabre et al., 2004). The early SR component described in this thesis is assumed to correspond to the first polysynaptic SR (late response) in rats with a transected spinal cord associated with the recovery of stepping ability (Lavrov et al., 2006).

The second important link between SR of animals and humans is the original cat work by Andén (1966) and Jankowska (1967). In the spinal cat an early and a late reflex was evoked, mediated through different neuronal pathways sharing a mutual inhibition. After an intravenous administration of L-DOPA, the early reflex was depressed, while a long-lasting, long-latency discharge was released instead (Jankowska et al., 1967). The long-latency discharges had a half-centre organisation, capable of generating alternating activation of extensors and flexors (Jankowska et al., 1967). Accordingly they were assumed to represent an activation of spinal locomotor circuitries (Forssberg and Grillner, 1973, Grillner and Zangger, 1979). It is assumed that this long-latency discharge corresponds to the early SR component described in this thesis due to association of both reflex components with locomotor ability after an SCI.

The late SR component found in chronic complete SCI, severely affected incomplete SCI and stroke subjects, has so far no physiological correlate in chronic animal models (see 7.4 Limitations).

7.3 Clinical significance

The aim of future regeneration-inducing therapies has to be to induce some regeneration of damaged fibre tracts before a neuronal dysfunction at lower levels is established. Therefore, the success of future approaches for regaining locomotor capacity after SCI or stroke will depend on the preservation of spinal neuronal function below the level of lesion. Functional training will certainly have a crucial role in this regard. Presently, regeneration-inducing therapies can hardly be successful in chronic complete SCI subjects if a neuronal dysfunction is established. The EMG exhaustion phenomenon and associated late SR components could not yet be reversed by a locomotor training in the chronic stage of such severely affected SCI subjects (see study 2 and (Dietz and Muller, 2004)). However, the second study has highlighted that intensive locomotor training with appropriate afferent input to spinal locomotor circuits is more successful to modify neuronal dysfunction in incomplete SCI subjects. In addition, regular training combined with enhanced afferent input by FES to spinal neuronal circuits seems to reverse neuronal dysfunction and is able to regain better neuronal function. Locomotor training combined with FES therapy might be a more effective way to exploit neuronal plasticity on spinal or even supraspinal levels than training therapy alone. Correspondingly, nice recovery of upper-limb function in stroke subjects is achieved by providing appropriate input to spinal neuronal circuitries, i.e., with combined repetitive transcranial magnetic stimulation and movement training (Koganemaru et al., 2010).

This thesis revealed that SR can serve as an electrophysiological marker for the functional state of spinal locomotor circuitries and can be used as a new tool to assess changes within these neuronal circuits. The SR might be used in addition to clinical assessments in SCI subjects to estimate the function of neuronal circuits, e.g., in psychogenic plegia or plegia due to biomechanical rather than neuronal constraints.

Despite the presence of common clinical characteristics of SCI and stroke, the fourth study provided evidence for lesion-dependent effects, i.e., what kind of loss of supraspinal drive leads to dysfunction of spinal neuronal circuits over time post-injury. In stroke subjects the impaired leg function was associated with a dominant late SR component (as in SCI subjects). However, changes in SR are not associated with an

exhaustion of leg muscle activity during assisted walking, even when stepping movements are performed solely by the affected leg. The lack of EMG exhaustion might be explained by subliminal influences of the unaffected leg muscle activation on the affected side. This, together with some training effects on affected leg muscles, might prevent EMG exhaustion.

By applying a focused training on the affected leg, in a similar way to constraint-induced movement therapy of the affected arm in stroke subjects (Taub et al., 1999), the affected leg could become trained without any compensation by the unaffected leg. As a consequence of such a training approach, a neuronal dysfunction, i.e., dominant late SR components, might be avoidable in stroke subjects.

7.4 Limitations of the thesis

The term 'spinal reflex' has been used through this whole thesis and might be questioned with regard to the literature. In general, a reflex response is described as an EMG response in the ipsilateral muscle with a fixed latency evoked by mechanical or electrical stimulation of the skin, a muscle or a nerve. If the reflex can be evoked in complete SCI subjects it has to be mediated by spinal pathways. If the latency is short (< 120 ms for the legs) in incomplete SCI and stroke subjects, it can be attributed to a SR. Our SR is of polysynaptic origin, as it has a longer latency than the H-reflex and is evoked in a different way. Study 1 included only complete SCI subjects with absent supraspinal input and therefore the term 'spinal reflex' is justified for both, the early and the late SR component. In addition, according to the perception in healthy subjects non-noxious stimuli were applied to the tibial nerve and therefore, one would not expect cortical responses due to pain perception. However, the origin and pathway of the late SR component in incomplete SCI and stroke subjects described in this thesis is less clear. It has to be pointed out that in these cases a possible contribution of supraspinal pathways, including either the brainstem or the cortex, or both can hardly be excluded. Nevertheless, according to the similar behaviour in complete and incomplete SCI subjects as well as the short onset latencies, at least the early SR component should be mediated on a spinal level.

Another limitation of this thesis represents the comparison of SR behaviour between animal models, namely rat and cat, and humans. The limitation concerns the different modes of stimulation used. In the study of Lavrov et al. (2006) SR were elicited by epidural stimulation in rats, while in our subjects the peripheral tibial nerve was

stimulated. In the cat model of Jankowska (1967) SR were evoked by electrical stimulation of flexion reflex afferents (FRA) and were directly recorded with microelectrodes from interneurons in the spinal segments. In addition, recordings were done under pharmacological interventions, i.e., the administration of L-DOPA. Nevertheless, from a functional point of view regarding the SR behaviour we would suggest that comparisons between animal models and humans are justified.

We have compared the early SR component described in this thesis on a physiological basis with animal models. However, for the late SR component found in chronic complete, severely affected incomplete and stroke subjects no chronic animal model is available so far. In addition, no condition in animals is known that corresponds to the human phenomenon of EMG exhaustion found in chronic complete SCI subjects. Another difficulty one would deal with is that in chronic incomplete rodent models spinal neurons might not become deprived of their appropriate supraspinal and peripheral input due to an automatic self-training of paralysed limbs which does not occur in human SCI. Self-trained rats had namely facilitated their locomotor activity by this automatic self-training (Edgerton et al., 1997).

There is a tendency in literature to group all cutaneous afferents together, and this might create confusion. However, a specific stimulation of defined afferents is hardly feasible in human beings. There is heterogeneity in: (i) the type of receptor (ii) the peripheral afferents, (iii) the spinal pathways fed by the afferents (number of interneurons), and (iv) their functional role (Pierrot-Deseilligny and Burke, 2005). Therefore, it is difficult to determine the specific afferents mediating the SR described in this thesis and how far they are comparable to other studies. Nevertheless, we assume that our SR components are mediated by low-threshold cutaneous afferents as described in the studies of Duysens et al. (2004), i.e., group II afferents. Certainly, no withdrawal reflexes, mediated by noxious nerve stimulation, were evoked.

7.5 Conclusion

A severe SCI leads to a dysfunction of spinal neuronal circuits underlying locomotor activity and associated reflexes. The neuronal dysfunction is reflected in a shift from dominant early to dominant late SR components and an associated EMG exhaustion during the time course of a complete SCI. In this thesis it became explored that rather the loss of afferent input to spinal neurons due to immobility after SCI than the completeness of the SCI plays a crucial role in the development of the spinal neuronal

dysfunction. In addition, SR can be used as a marker for the functional state of spinal locomotor circuitry. In incomplete SCI subjects the pathological changes of this marker for a neuronal dysfunction are not irreversible, but modifiable by a locomotor training. In incomplete SCI subjects first approaches have been proposed to overcome a spinal neuronal dysfunction with an appropriate combination of intensive locomotor training and FES.

In contrast to SCI subjects, the neuronal dysfunction in stroke subjects has a differential appearance and impact. Although the two different central nervous lesions share common clinical symptoms, stroke subjects seem to benefit from the positive influence of the unaffected to the affected side and the remaining supraspinal input.

The basic mechanisms underlying the spinal neuronal dysfunction are not yet clear. We hypothesise that the loss of supraspinal and appropriate afferent input to spinal neuronal circuitries after an SCI leads to weakened function of excitatory interneuronal circuits involved in the generation of a locomotor pattern. An important point for future SCI therapy approaches is to overcome the dysfunction of spinal neuronal circuits below the level of lesion by appropriate functional training. Without a preservation of these circuits regeneration-inducing therapies might fail to be successful.

7.6 Outlook

This thesis answered some questions concerning changes in the function of spinal neuronal circuitries after a central nervous lesion, but concurrently opened several new questions. Further research is required to more precisely define the nature of the neuronal dysfunction, i.e., the changes of excitatory and inhibitory activity of neuronal circuits below the level of lesion. In addition, studies on an animal model of chronic SCI might be able to elucidate the mechanisms underlying the neuronal dysfunction after an SCI / stroke and facilitates the development of appropriate countermeasures.

Another important question to be solved will be: How can we gain best access to spinal neuronal networks in SCI subjects and train them appropriately to prevent a neuronal dysfunction below the level of lesion? Partial responses to those questions can be deduced from the findings of study 2. Functional improvements and associated strengthening of the early SR component were found in incomplete SCI subjects after an intensive locomotor training. The best functional and electrophysiological outcome was gained by a subject trained with combined treadmill training and FES. Therefore, we assume that enhancing of afferent input to spinal neuronal circuitries might prevent a

neuronal dysfunction. In future, locomotor training should be optimised by enhancing afferent input not only by load and hip joint receptors (Dietz et al., 2002), but also by providing enhanced afferent input. This enhanced afferent input could be provided by electrical stimulation of FRA or cutaneous afferents, to repetitively induce the early SR component, alone or in combination with limb movements.

Another approach to gain access to spinal neuronal circuitries is electrical stimulation of the spinal cord. So far, epidural electrical stimulation of the lumbar spinal cord in combination with pharmacological interventions in rats with a complete spinal cord transection could regain full weight-bearing locomotion (Courtine et al., 2009). In addition, invasive epidural stimulation could induce patterned leg muscle activity in complete SCI subjects (Dimitrijevic et al., 1998, Minassian et al., 2004). In contrast to the invasive epidural stimulation, the transcutaneous spinal direct current stimulation (tsDCS) is a non-invasive stimulation of the spinal cord applied through surface electrodes. This method is deduced from the transcranial direct current stimulation (tDCS) which has been used for several decades to influence specific brain areas (Nitsche et al., 2008). The transfer of this method from the brain to the spinal cord is a new approach. TsDCS can modulate spinal neuronal circuitries, i.e., can modify H- and F-reflex responses or somato-sensory evoked potentials in healthy subjects (Cogiamanian et al., 2008, Cogiamanian et al., 2010, Winkler et al., 2010). Therefore, it should be aimed to investigate whether tsDCS can enhance the functional state of spinal neuronal circuits. A combination of tsDCS with a locomotor training might promote neuronal plasticity with the consequence of regaining better functional outcome, reflected in dominant early SR components in incomplete SCI subjects.

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9 Abbrevations

AIS	ASIA impairment scale
ASIA	American Spinal Injury Association
BF	Biceps femoris muscle
BWS	Body weight support
C	Cervical vertebral body
cm	Centimeter
CI	Confidence interval
CPG	Central pattern generator
cSCI	Complete spinal cord injury
DGO	Driven gait orthosis
EMG	Electromyogramm
FAC	Functional ambulation category
GM	Gastrocnemius medialis muscle
Hz	Hertz
L-DOPA	L-3,4-Dihydroxyphenylalanin
mA	Milliampere
MI	Motricity index
min	Minutes
mm	Milimeter
ms	Millisecond
MT	Motor threshold
µV	Microvolt
OEC	Olfactory ensheathing cell
RF	Rectus femoris muscle
RMI	Rivermead mobility index
RMS	Root mean square
RT	Reflex threshold
SCI	Spinal cord injury
SCIM	Spinal cord independence measure
SD	Standard deviation
sec	Second
SR	Spinal reflex

T	Thoracic vertebral body
TA	Tibialis anterior muscle
WISCI	Walking index for spinal cord injury
10MWT	10 meter walking test

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